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## TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Application Number	10/015,390
Filing Date	December 12, 2001
First Named Inventor	David Botstein
Group/Art Unit	1637
Examiner Name	Jeffrey Norman Fredman

Total Number of Pages in This Submission

194

Attorney Docket Number

39780-2830 P1C53

### ENCLOSURES (check all that apply)

☐ Fee Transmittal Form

☐ Fee Attached

☒ Appellants' Brief

☒ After Final

☐ Version With Markings Showing  
Changes

☐ Affidavits/declaration(s)

☐ Extension of Time Request

☐ Information Disclosure Statement

☐ Certified Copy of Priority Document(s)

☐ Response to Missing Parts/ Incomplete  
Application

☐ Response to Missing  
Parts under 37 CFR  
1.52 or 1.53

☐ Copy of Notice

☐ Copy of an Assignment

☐ Drawing(s)

☐ Licensing-related Papers

☐ Petition Routing Slip (PTO/SB/69)  
and Accompanying Petition

☐ Petition to Convert to a  
Provisional Application

☐ Power of Attorney, by Assignee to  
Exclusion of Inventor Under 37 C.F.R.  
§3.71 With Revocation of Prior Powers

☐ Terminal Disclaimer

☐ Small Entity Statement

☐ Request for Refund

☐ After Allowance Communication to  
Group

☐ Appeal Communication to Board of  
Appeals and Interferences

☒ Appeal Communication to Group  
(Appeal Notice, Brief, Reply Brief)

☐ Proprietary Information

☐ Status Letter

☒ ADDITIONAL ENCLOSURE(S)  
(PLEASE IDENTIFY BELOW):

☒ Evidence Appendix Items 1 thru 5;  
and return postcard.

Remarks

**AUTHORIZATION TO CHARGE DEPOSIT ACCOUNT 08-1641 FOR ANY FEES DUE IN  
CONNECTION WITH THIS PAPER, REFERENCING ATTORNEY'S DOCKET NO. 39780-  
2830P1C53.**

### SIGNATURE OF APPLICANT, ATTORNEY OR AGENT

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Date	AUGUST 15, 2005	Customer Number: 35489

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8-17-05

AF/1637  
DAW

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	)	Examiner: Fredman, Jeffrey Norman
	)	
David BOTSTEIN, et al.	)	Art Unit: 1637
	)	
Application Serial No. 10/015,390	)	Confirmation No: 9959
	)	
Filed: December 12, 2001	)	Attorney's Docket No. 39780-2830 P1C53
	)	
For: <b>SECRETED AND</b>	)	<b>Customer No. 35489</b>
<b>TRANSMEMBRANE</b>	)	
<b>POLYPEPTIDES AND NUCLEIC</b>	)	
<b>ACIDS ENCODING THE SAME</b>	)	

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**ON APPEAL TO THE BOARD OF PATENT APPEALS AND INTERFERENCES**  
**APPELLANTS' AMENDED BRIEF IN RESPONSE TO NOTICE OF NON-**  
**COMPLIANT APPEAL BRIEF**

**MAIL STOP APPEAL BRIEF - PATENTS**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, Virginia 22313-1450

Dear Sir:

On November 8, 2004, the Examiner made a final rejection to pending Claims 33, 38-40 and 44-47. A Notice of Appeal was filed on March 2, 2005. Appellants' Brief on Appeal was filed July 1, 2005.

A Notification of Non-Compliant Appeal Brief was mailed July 20, 2005, which stated that the brief was defective because incorrect headings were used, and several required elements were missing. These defects have been corrected in the following amended appeal brief.

The following constitutes the amended version of Appellants' Brief on Appeal.

**1. REAL PARTY IN INTEREST**

The real party in interest is Genentech, Inc., South San Francisco, California, by an assignment of the patent application U.S. Serial No. 09/946,374 recorded January 8, 2002, at Reel 012288 and Frame 0504.

**2. RELATED APPEALS AND INTERFERENCES**

There are no related appeals or interferences known to Appellants, Appellants' legal representative, or Appellants' assignee that will directly affect or be directly affected by or have a bearing on the Board's decision in the present appeal.

**3. STATUS OF CLAIMS**

Claims 33, 38-40, and 44-47 are in this application.

Claims 1-32, 34-37, 41-43, and 48-54 are canceled.

Claims 33, 38-40, and 44-47 stand rejected and Appellants appeal the rejection of these claims.

A copy of the rejected claims involved in the present Appeal is provided as Appendix A.

**4. STATUS OF AMENDMENTS**

Claims 48-54 were canceled by amendment in the Response to Office Action filed January 7, 2005. This amendment was entered according to the Advisory Action mailed February 15, 2005.

**5. SUMMARY OF CLAIMED SUBJECT MATTER**

The invention claimed in the present application is related to an isolated nucleic acid comprising the nucleic acid sequence of SEQ ID NO:215; the full-length coding sequence from within the nucleic acid sequence of SEQ ID NO:215; or the full-length coding sequence of the cDNA deposited under ATCC accession number 203226 (Claims 33, 38, 39, and 40); a vector comprising the nucleic acid (Claims 44 and 45); and a host cell comprising the nucleic acid (Claims 46 and 47).

The cDNA nucleic acid encoding PRO1269 is described in the specification at page 154, lines 10-14, in Figure 121 and in SEQ ID NO:215. The full-length PRO1269 polypeptide is described in the specification at, for example, page 432, lines 1-11, in Figure 122 and in SEQ ID NO:216. Page 292, lines 14-18 of the specification provides the description for Figures 121 and 122. That the PRO1269 sequence has homology to granulocyte peptide A is disclosed in the specification at page 17, lines 19-24 and at page 344, lines 11-17. Methods for isolating PRO cDNA is generally set forth in the specification at, for example page 359, lines 9-34. Methods for selection and transformation of host cells with PRO cDNA is generally set forth in the specification at, for example, page 359, line 36, to page 361, line 24. Methods for selecting a vector are generally set forth in the specification at, for example, page 361, line 26, to page 363, line 25. In particular the isolation of cDNA clones encoding PRO1269 is set forth in the specification in Example 64, at page 431, line 26, to page 432, line 13. Finally, Example 143, in the specification at page 494, line 20, to page 508, line 28, sets forth a Gene Amplification assay which shows that the PRO1269 gene is amplified in the genome of certain human lung cancers (see page 505, lines 23-30).

## **6. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL**

I. Whether Claims 33, 38-40, and 44-47 are patentable under 35 USC § 102(e) over Young *et al.*, U.S. Patent No. 6,444,790.

## **7. ARGUMENT**

Claims 33, 38-40 and 44-47 stand rejected under 35 U.S.C. §102(e) as allegedly being anticipated by Young *et al.*, (U.S. Patent No. 6,444,790, effective priority date December 23, 1998).

Appellants have claimed priority to U.S. Provisional Application No. 60/100,661, filed on September 16, 1998. The present application is entitled to the priority date of September 16, 1998, which precedes, by at least three months, the earliest priority date of Young *et al.*

(December 23, 1998). Accordingly, Young *et al.* is not prior art against the present application and Claims 33, 37-40 and 44-47 are patentable.

Appellants have previously submitted signed copies of a Declaration under 37 C.F.R. §1.131 by Dr. Botstein, Dr. Goddard, Dr. Godowski, Dr. Gurney, Ms. Roy, Mr. Watanabe and Dr. Wood on November 23, 2004, that establishes that Appellants had cloned, sequenced and identified homology to granulocyte peptide A for the claimed sequences before the prior art date of December 23, 1998.

In order to remove a reference as a prior art, “[i]t is sufficient if [the affidavit under Patent Office Rule 131] shows that as much of the claimed invention as is taught in the reference has been reduced to practice by the [patentee] prior to the date of the reference.” *In re Stempel*, 241 F.2d 755, 757 (1957). *In re Moore*, 170 U.S.P.Q. 260 (C.C.P.A. 1971), confirmed the holding in *In re Stempel*, adding that “the determination of a practical utility when one is not obvious need not have been accomplished prior to the date of a reference unless the reference also teaches how to use the compound it describes.” *In re Moore*, 170 U.S.P.Q. at 267 (emphasis added). Accordingly, Appellants respectfully submit that in order to overcome the 35 U.S.C. §102(e) rejection over Young *et al.*, the Declaration simply needs to provide a disclosure commensurate in scope with the disclosure in the prior art document by Young *et al.* to support the priority claim.

The cited reference by Young *et al.* discloses a polypeptide sequence designated peptidoglycan recognition protein-related proteins-chondrosarcoma (PGRP-C), which is identical to the PRO1269 polypeptide of the present application. The cited reference further discloses the encoding nucleic acid sequence for the PGRP-C and sequence homology with both human peptidoglycan recognition protein (PGRP) and murine Tag-7, but is devoid of any experimental data demonstrating the biological activity of PGRP-C, or identifying any specific diseases associated with the expression level of this protein or its encoding gene. Thus Appellants respectfully submit that the Declaration simply needs to show possession of the polypeptide sequence, its encoding polynucleotide sequence as disclosed in Young *et al.*, and a sequence homology in order to overcome the 35 U.S.C. §102 rejection.

Accordingly, Appellants respectfully submit that the disclosures are commensurate in scope and that U.S. Provisional Application No. 60/100,661, filed on September 16, 1998, discloses all that the cited prior art discloses. Consequently, based on the holdings of *In re Stempel* and *In re Moore*, Appellants respectfully submit that Young *et al.* is not prior art under 102(e) since its effective priority date is after the invention by the Appellants for patent.

The Examiner has asserted that the standard set forth in *In re Stempel* and *In re Moore* does not apply because Young *et al.* allegedly teach a use for PGRP-C (SEQ ID NO:6). The Examiner's presumption of utility for SEQ ID NO:6 based upon the fact that the Young patent issued is incorrect, because the issued claims of the Young *et al.* patent are directed to SEQ ID NO:4 and not SEQ ID NO:6. The utilities of SEQ ID NO:4 in the diagnosis of specific disorders such as wound healing cannot be imputed to SEQ ID NO:6, because in contrast to SEQ ID NO:4, SEQ ID NO:6 is not shown or stated to be expressed in wound healing tissues. Nor does the disclosure that nucleic acids encoding PGRP-C were found in cDNA libraries derived from human chondrosarcoma, without more, suffice to provide utility for PGRP-C, because there is no evidence from Young *et al.* that SEQ ID NO:6 is overexpressed in chondrosarcoma as compared to any control sample. The statements in the Young patent regarding differential expression of PGRP-C in diseased tissues are purely speculative. This does not suffice to enable one of ordinary skill in the art to use the disclosed PGRP-C sequence in the diagnosis of any diseases.

Finally, while Appellants' priority document is not required to provide utility for PRO1269, U.S. Provisional Application Serial No. 60/100,661 in fact does teach a utility for the claimed PRO1269 sequence based upon homology to known granulocyte peptide A precursors.

As disclosed in the specification of U.S. Provisional Application Serial No. 60/100,661, the amino acid sequence of PRO1269 (SEQ ID NO:1) has about 70% amino acid sequence identity to the bovine granulocyte peptide A precursor (page 15, lines 14-17). The specification further disclosed known utilities for members of the granulocyte peptide A family, based upon the Selsted published patent application WO 97/29765, which was incorporated by reference into the application as filed, and made of record in the Information Disclosure Statement submitted November 7, 2002. The Selsted application, published on August 21, 1997, clearly teaches specific, substantial, and credible utilities for the bovine and mouse granulocyte A peptide family

members, including potent antimicrobial, antiviral, antiprotozoal, and antifungal activities. Thus the utility of granulocyte A peptides had been clearly demonstrated at the time of filing of U.S. Provisional Application Serial No. 60/100,661. Moreover, an application containing the identical text as the Selsted WO 97/29765 application subsequently issued as U.S. Patent No. 6,696,559, on February 24, 2004, containing claims directed to isolated nucleic acid sequences encoding the bovine and mouse granulocyte A peptide precursor sequences.

As explained in the M.P.E.P. § 2107.03, the courts "have routinely found evidence of structural similarity to a compound known to have a particular therapeutic or pharmacological utility as being supportive of an assertion of therapeutic utility for a new compound." See also *In re Jolles*, 628 F.2d 1322, 206 U.S.P.Q. 885 (C.C.P.A. 1980), and *In re Brana*, 51 F.3d 1560, 1566, 34 U.S.P.Q.2d 1436, 1441 (Fed. Cir. 1995). The claimed PRO1269 sequence displays significant structural similarity to the known useful granulocyte peptide A compounds, based upon both overall sequence identity and the conservation of important functional residues. Accordingly, based upon the standard set forth in *In re Jolles* and *In re Brana*, the priority application disclosed a patentable utility for PRO1269.

These arguments are discussed in further detail below.

**A. U.S. Provisional Application Serial No. 60/100,661 simply needs to disclose what is disclosed in the cited reference to support the priority claim**

Appellants respectfully submit that in order to overcome the 35 U.S.C. §102(e) rejection over Young *et al.*, the Declaration by Dr. Botstein, Dr. Goddard, Dr. Godowski, Dr. Gurney, Ms. Roy, Mr. Watanabe and Dr. Wood ("Declaration") simply needs to provide a disclosure commensurate in scope with the disclosure in the prior art document by Young *et al.* to support the priority claim.

In order to remove a reference as a prior art, "[i]t is sufficient if [the affidavit under Patent Office Rule 131] shows that as much of the claimed invention as is taught in the reference has been reduced to practice by the [patentee] prior to the date of the reference." *In re Stempel*, 241 F.2d 755, 757 (1957). In *In re Stempel*, the patent applicant (Stempel) had claims directed to both (i) a particular genus of chemical compounds (the "generic" claim) and (ii) a single species of chemical compound that was encompassed within that genus (the "species" claim). In support

of a rejection under 35 U.S.C. §102, the examiner cited against the application a prior art reference that disclosed the exact chemical compound recited in the “species” claim. In response to the rejection, the patent applicant filed a declaration under 37 C.F.R. §1.131 demonstrating that he had made that specific chemical compound prior to the effective date of the cited prior art reference. The Court found the applicant’s 37 C.F.R. § 1.131 declaration effective for swearing behind the cited reference for purposes of both the “species” claim and the “genus” claim. Specifically, the Court stated in support of its decision that “all the applicant can be required to show is priority with respect to so much of the claimed invention as the reference happens to show. When he has done that he has disposed of the reference.” *Id.* at 759.

Furthermore, the Board's attention is respectfully directed to *In re Moore*, 170 U.S.P.Q. 260 (C.C.P.A. 1971), where the holding in *In re Stempel* was affirmed. In *In re Moore*, the patent applicant claimed a particular chemical compound in his patent application and the examiner cited against the applicant a prior art reference under 35 U.S.C. §102 rejection which disclosed the compound but did not disclose any specific utility for the compound. The patent applicant filed a declaration under 37 C.F.R. §1.131 demonstrating that he had made the claimed compound before the effective date of the cited prior art reference, even though he had not yet established a utility for that compound. On appeal, the Court indicated that the 131 declaration filed by the patent applicant was sufficient to remove the cited reference. The Court relied on the established “Stempel Doctrine” to support its decision, stating:

An applicant need not be required to show [in a declaration under 37 C.F.R. § 1.131] any more acts with regard to the subject matter claimed that can be carried out by one of ordinary skill in the pertinent art following the description contained in the reference ... the determination of a practical utility when one is not obvious need not have been accomplished prior to the date of a reference unless the reference also teaches how to use the compound it describes.

*In re Moore*, 170 U.S.P.Q. at 267 (emphasis added).

Thus, *In re Moore* confirmed the holding in *In re Stempel* which states that in order to effectively remove a cited reference with a declaration under 37 C.F.R. §1.131, an applicant need only show that portion of his or her claimed invention that appears in the cited reference.

Young *et al.* discloses a protein designated peptidoglycan recognition protein-related proteins-chondrosarcoma (PGRP-C), which is identical to the PRO1269 polypeptide of the present application. The specification discloses that PGRP-C has sequence homology with both human peptidoglycan recognition protein (PGRP) and murine Tag-7 as support for the sequence possibly being useful in augmenting the immune system in areas such as immune recognition and immune system activation. (See U.S. Patent No. 6,444,790, column 1 lines 16-21; column 3, lines 38-46; column 60, lines 56-67; column 61, lines 15-53). However, the specification of the issued U.S. patent is devoid of any experimental data demonstrating the biological activity of PGRP-C, or identifying any specific diseases associated with the expression level of this protein or its encoding gene.

Accordingly, since the cited reference by Young *et al.* only discloses a polypeptide sequence, its encoding nucleic acid sequence and a sequence homology, Appellants respectfully submit that the Declaration simply needs to show possession of the polypeptide sequence, its encoding polynucleotide sequence as disclosed in Young *et al.*, and a sequence homology in order to overcome the 35 U.S.C. §102 rejection.

Appellants have respectfully submitted that U.S. Provisional Application No. 60/100,661, filed on September 16, 1998, provides the nucleic acid and amino acid sequences of the PRO1269 polypeptide and the homology of the polypeptide to the bovine granulocyte peptide A precursor (see U.S. Provisional Application No. 60/100,661 on page 15, under the section titled "Full-length PRO1269").

The Declaration clearly states that U.S. Provisional Application No. 60/100,661, filed on September 16, 1998, discloses sequences designated as SEQ ID NO:2 and SEQ ID NO:1, which are identical to SEQ ID NO:215 and SEQ ID NO:216, respectively, of the above-identified application. Further, the Declaration confirms that U.S. Provisional Application No. 60/100,661, filed on September 16, 1998, discloses that SEQ ID NO:1, corresponding to SEQ ID NO: 216 of the above-identified application, has homology to granulocyte peptide A.

Accordingly, Appellants respectfully submit that the disclosures are commensurate in scope and that U.S. Provisional Application No. 60/100,661, filed on September 16, 1998, discloses all that the cited prior art discloses.

Consequently, based on the holdings of *In re Stempel* and *In re Moore*, Appellants respectfully submit that *Young et al.* is not prior art under 102(e) since its effective priority date is after the invention by the Appellants for patent.

**B. Young et al. does not teach a utility for SEQ ID NO:6**

The Examiner has contended that Stempel "states in relevant part 'unless the reference also teaches how to use the compound it describes (see page [12] of the response).' This is precisely that situation. It is undisputed, and actually admitted by Applicant, that their U.S. Provisional Application Serial No. 60/100,661 does not provide any utility for the claimed sequence." (Pages 12-13 of the Office Action mailed November 8, 2004). Furthermore, the Examiner has asserted that "Young is a reference that also teaches how to use the compound it describes.... The Young patent is literally identical to the provisional from which it depends (U.S. Provisional Application Serial No. 60/113,809). The Young patent provides identical utilities for the claimed SEQ ID NO:4 and for the sequence at issue, SEQ ID NO:6. Since issued patents are PRESUMED useful and enabled, and no evidence overcoming that presumption has been presented, Young is presumptively enabled for SEQ ID NO:6 simply based on the fact that the patent issued." (Page 13 of the Office Action mailed November 8, 2004).

Appellants respectfully submit that the Examiner's conclusions concerning the presumption of utility and enablement for SEQ ID NO:6 in U.S. Patent No. 6,444,790 (*Young et al.*) "simply based on the fact that the patent issued" are flawed for several reasons. First of all, as the Examiner has admitted above, the issued claims of the *Young et al.* patent are directed to isolated proteins comprising various amino acid residues of SEQ ID NO:4 and not SEQ ID NO:6. Since the presumption of validity applies only to the subject matter covered by the claims of an issued patent, contrary to the Examiner's assertion, the utility for SEQ ID NO:6 cannot be presumed based on the fact that the *Young et al.* patent issued with claims covering proteins other than SEQ ID NO:6. Secondly, even if the patent had issued with claims covering SEQ ID NO:6 (as it had not), the presumption of validity would be rebutted by the fact that the disclosure of *Young et al.* is completely devoid of any teaching of a real life utility for this molecule.

The Examiner has asserted that "Young teaches specific diagnosis of specific disorders including wound healing at column 6, lines 48-67. This is a specific and substantial utility, unlike those presented in the current application.... The specification expressly states this diagnostic ability and the differential expression of the protein during wound healing. Diagnosing problems in wound healing is clearly a credible, specific and substantial utility." (Page 13 of the Office Action mailed November 8, 2004). Appellants respectfully point out to the that the Young patent teaches three peptidoglycan recognition protein-related proteins expressed by keratinocytes, wound-healing tissues and chondrosarcoma tissue, referred to as PGRP-K (Keratinocytes), PGRP-W (Wound-healing) and PGRP-C (Chondrosarcoma), respectively. (See Abstract). The amino acid sequence of PGRP-K is shown in SEQ ID NO:2, the amino acid sequence of PGRP-W is shown in SEQ ID NO:4 and the amino acid sequence of PGRP-C is shown in SEQ ID NO:6. More specifically, the Young *et al.* patent teaches that:

the nucleic acid molecule described in FIG. 1 (SEQ ID NO:1) was discovered in a cDNA library derived from Human keratinocytes, the nucleic acid molecule described in FIG. 2 (SEQ ID NO:3) was discovered in cDNA libraries derived from Human keratinocytes and Human tissues undergoing wound-healing, and the nucleic acid molecule described in FIG. 3 (SEQ ID NO:5) was discovered in cDNA libraries derived from Human chondrosarcoma. (Column 9, lines 40-48).

Therefore, a careful reading of the Young *et al.* patent shows that comments relating to various disorders wherein a higher or lower levels of the gene expression may be detected in the wound healing tissues are specifically directed to PGRP-W, SEQ ID NO:4 and not PGRP-C, SEQ ID NO:6, because SEQ ID NO:6 is not shown or stated to be expressed in wound healing tissues.

In addition, Appellants note that the Young *et al.* patent teaches, "PGRP-W is 42% homologous to PGRP-C, and PGRP-K is 39% homologous to PGRP-C." (See column 10, lines 8-9 of U.S. Patent No. 6,444,790). Therefore, it would appear that PGRP-C has rather low sequence identity to both PGRP-W and PGRP-K. Thus, based solely on homology, a person skilled in the art at the priority date of that application would not have reasonably concluded that PGRP-W and PGRP-C would have the same utility.

Hence, the disclosure for "diagnosing problems in wound healing" in the Young *et al.* patent does not support a diagnostic utility for the sequence at issue, SEQ ID NO:6.

Nor does the disclosure that nucleic acids encoding PGRP-C were found in cDNA libraries derived from human chondrosarcoma, without more, suffice to provide utility for PGRP-C. Appellants respectfully point out that many genes may be found in chondrosarcomas or other tumors. Those useful as markers are those which are overexpressed in the tumor as compared to control tissue. This is acknowledged in the Young patent (Column 61, lines 45-53), which states,

Thus, the invention provides a diagnostic method ...which involves assaying the expression level of the gene encoding the PGRP-K, PGRP-W and/or PGRP-C polypeptide(s) in mammalian cells or body fluid and comparing the gene expression level with a standard PGRP-K, PGRP-W and/or PGRP-C gene expression level, whereby an increase or decrease in the gene expression level over the standard is indicative of the disease.

Yet there is no evidence from Young *et al.* that SEQ ID NO:6 is overexpressed in chondrosarcoma as compared to a standard control sample; in fact, there is no indication that expression levels of SEQ ID NO:6 in any other tissues were ever examined. The mere fact that the gene encoding SEQ ID NO:6 happened to have been first isolated from cDNA libraries derived from chondrosarcoma does not in itself make SEQ ID NO:6 useful as a diagnostic marker for cancer, absent evidence that SEQ ID NO:6 is differentially expressed in chondrosarcoma as compared to control tissues.

The statements in the Young patent regarding differential expression of PGRP-C in diseased tissues are purely speculative. See, for example, Column 61, lines 16-28, which states,

Thus it is believed that certain tissues in mammals with certain diseases and infections...., diseases associated with increased or decreased cell survival, .... express significantly altered (e.g., *enhanced or decreased*) levels of either the PGRP-K, PGRP-W and/or PGRP-C polypeptides and mRNAs encoding the PGRP-K, PGRP-W and/or PGRP-C polypeptides when compared to a corresponding "standard" mammal.

(Emphasis added). Similarly, Column 60, lines 57-59 states, "Cells which express either the PGRP-K, PGRP-W and/or PGRP-C polypeptides are believed to have a potent cellular response to infection ...."

Appellants therefore maintain the position that the Young *et al.* patent is devoid of any experimental data demonstrating the biological activity of PGRP-C, or identifying any specific diseases associated with the expression level of this protein or its encoding gene. As mentioned above, while Young *et al.* discloses a protein designated PGRP-C and provides sequence homology to both human peptidoglycan recognition protein (PGRP) as well as murine Tag-7, it does not provide any specific experimental data to support the utility in diagnosing various disorders by assaying the PGRP-C gene expression levels. All of the teachings in the Young *et al.* patent regarding such utility is merely speculative and entirely dependent on the fact that the nucleic acid encoding the PGRP-C polypeptide happened to be first discovered in cDNA libraries derived from human chondrosarcoma. (See column 9, lines 45-48). Accordingly, the Young *et al.* patent is devoid of any experimental support that would show how PGRP-C can be used to diagnose any disorders or diseases. It merely suggests that PGRP-C may be useful in diagnosing certain disorders/diseases. This does not suffice to enable one of ordinary skill in the art to use the disclosed PGRP-C sequence.

**C. U.S. Provisional Application Serial No. 60/100,661 teaches a utility for PRO1269 based upon homology to known granulocyte peptide A precursors**

The Examiner has asserted that "[i]t is undisputed, and actually admitted by Applicant, that their U.S. Provisional Application Serial No. 60/100,661 does not provide any utility for the claimed sequence." (Page 13 of the Office Action mailed November 8, 2004). Appellants respectfully point out that this assertion is incorrect. Appellants have never stated that U.S. Provisional Application Serial No. 60/100,661 does not provide any utility for the claimed PRO1269 sequence. What Appellants have actually argued, as discussed above, is that because the cited Young *et al.* reference does not provide utility for the disclosed PGRP-C sequence (SEQ ID NO:6), U.S. Provisional Application Serial No. 60/100,661 is not required to provide utility for PRO1269, because the priority document need not show more than the cited reference does. However, while Appellants' priority document is not required to provide utility for PRO1269, it does in fact do so. The U.S. Provisional Application Serial No. 60/100,661 discloses utility for the claimed PRO1269 sequence as a member of the granulocyte peptide A family.

As disclosed in the specification of U.S. Provisional Application Serial No. 60/100,661, the amino acid sequence of PRO1269 (SEQ ID NO:1) has about 70% amino acid sequence identity to the bovine granulocyte peptide A precursor (page 15, lines 14-17). The specification further disclosed known utilities for members of the granulocyte peptide A family, noting that "[p]atent publication no. WO9729765-A1, to Selsted, describes the identification of granulocyte peptide A which was isolated from bovine and mouse granulocytes. Several uses for this peptide were identified including, a therapeutic use, use as an agricultural agent, use as a preservative for food, and use as a water treatment agent" (page 2, lines 11-15). The specification asserted that these utilities also applied to the claimed PRO1269 sequence, stating that "it is presently believed that PRO1269 disclosed in the present application is a newly identified member of the granulocyte A peptide family and may possess microbial activity typical of that family of peptides" (page 15, lines 20-24).

The Selsted published patent application WO 97/29765 was made of record in the Information Disclosure Statement submitted November 7, 2002. The Selsted application,

published on August 21, 1997, clearly teaches specific, substantial, and credible utilities for granulocyte A peptide family members. For example, the Selsted application states that the purified granulocyte A peptides "have potent antimicrobial, antiviral, antiprotozoal, and antifungal activities." (Page 3, lines 4-5). The Selsted application further states that these peptides "are effective compounds for use in human and/or veterinary medicine, or as agents in agricultural, food science, or industrial applications." (Page 3, lines 6-8). In addition, antimicrobial activity of the granulocyte A peptides against representative Gram positive and Gram negative bacteria, as well as the yeast forms of two fungi, was experimentally demonstrated (see Example 5, at page 39, lines 1-27). Thus the utility of granulocyte A peptides had been clearly demonstrated at the time of filing of U.S. Provisional Application Serial No. 60/100,661.

Appellants respectfully note that an application containing the identical text as the Selsted WO 97/29765 application subsequently issued as U.S. Patent No. 6,696,559, on February 24, 2004. The issued Selsted patent contains claims 4 and 5, directed to isolated nucleic acid sequences encoding SEQ ID NO:3 and SEQ ID NO:5, which are the bovine and mouse granulocyte A peptide precursor sequences. Since, as stated by the Examiner, issued patents are presumed useful and enabled, and no evidence overcoming that presumption has been presented, it may be presumed from the issuance of the Selsted patent that nucleic acids encoding granulocyte A peptide precursor sequences are useful and enabled.

The Selsted WO 97/29765 application and issued U.S. Patent No. 6,696,559 are directed to bovine and mouse granulocyte A peptides and precursor sequences. One of ordinary skill in the art would have readily understood at the time of filing that the human granulocyte A peptide precursor, PRO1269, would share the same utilities as the bovine and mouse homologs. As stated in U.S. Provisional Application Serial No. 60/100,661, PRO1269 has about 70% amino acid sequence identity to the bovine granulocyte A peptide precursor. The sequence identity between the bovine and mouse proteins disclosed in the Selsted application is only 55% (see the enclosed sequence alignment; Exhibit A), but the Selsted application notes that "this gene family appears to be remarkably conserved" (page 6, lines 18-19), and both the bovine and mouse proteins were found to have the same function. Thus the sequence identity of 70% between the

human and bovine proteins easily meets a standard recognized in the art as indicative of conserved function. Moreover, the Selsted application identified conserved residues in the C-terminal region of the protein, which comprises the active peptide (page 8, lines 23-27). As shown in Figure 6 of Selsted, both the bovine and mouse peptides have a conserved C-terminal motif of YXXIQXWXHYR. All of these conserved residues are also found in the amino acid sequence of PRO1269, SEQ ID NO:216 (see Figure 122 of the instant application, and Figure 1 of the 60/100,661 priority application). Thus one of ordinary skill in the art would reasonably have expected the human protein to have the same activity as the bovine and mouse homologs.

As explained in the M.P.E.P. § 2107.03, the courts "have routinely found evidence of structural similarity to a compound known to have a particular therapeutic or pharmacological utility as being supportive of an assertion of therapeutic utility for a new compound." For example, in *In re Jolles*, 628 F.2d 1322, 206 U.S.P.Q. 885 (C.C.P.A. 1980), the claimed compounds were found to have utility based on a finding of a close structural relationship to daunorubicin and doxorubicin, both of which were known to be useful in cancer chemotherapy. Similarly, in *In re Brana*, 51 F.3d 1560, 1566, 34 U.S.P.Q.2d 1436, 1441 (Fed. Cir. 1995), in which the court declared the rejection of claims improper where the claims did "not suggest an inherently unbelievable undertaking or involve implausible scientific principles" and where "prior art . . .discloses structurally similar compounds to those claimed by applicants which have been proven. . . .to be effective."

As discussed above, the bovine and mouse members of the granulocyte peptide A family were known at the time of filing for the priority application to have utility based upon their antimicrobial activity. The claimed PRO1269 sequence displays significant structural similarity to these known useful compounds, based upon both overall sequence identity and the conservation of important functional residues. Accordingly, based upon the standard set forth in *In re Jolles* and *In re Brana*, the priority application disclosed a patentable utility for PRO1269.

U.S. Provisional Application Serial No. 60/100,661 further discloses how to make and used claimed polynucleotide sequences encoding PRO1269. The provisional specification provides in the method used to identify and clone the PRO1269 nucleic acid sequence (Example 1, at page 47, line 31, to page 49, line 31). The specification provides other methods which

could be used to obtain the PRO1269 polynucleotide (page 22, line 1, to page 23, line 15). The specification provides methods which could be used for selecting and using a vector for the expression of PRO1269 (page 25, line 20, to page 29, line 30) and methods which could be used for selecting and transforming host cells with PRO1269 (page 23, line 16, to page 24, line 19). The specification sets forth a number of different uses for the nucleotide sequences encoding PRO1269 polypeptides at, for example, pages 31-36. Such uses include use of the PRO1269 polypeptide in pharmaceutical compositions (page 36, lines 29-33). One of ordinary skill in the art would further understand how to make and use PRO1269 based upon the disclosure of the Selsted patent application, which was expressly incorporated by reference in its entirety (page 47, lines 15-17).

Accordingly, Appellants respectfully submit that even if the cited Young *et al.* patent discloses a utility for SEQ ID NO:6 (which is expressly not conceded) the disclosures are still commensurate in scope, and U.S. Provisional Application No. 60/100,661, filed on September 16, 1998, discloses all that the cited prior art discloses. For this reason, Young *et al.* is not prior art against the present application and the rejection of claims 33, 38-40 and 44-47 under 35 U.S.C. §102(e) as allegedly being anticipated by Young *et al.* should be reversed.

Finally, Appellants submit that they have provided in U.S. Patent Application No. 60/100,661 a disclosure very similar to that of Young *et al.* Young *et al.* was granted a patent based on its disclosure. Appellants submit that they are unfairly being held to a different standard of patentability than that applied to the Young *et al.* patent application. It is legally wrong and inequitable to hold Appellants to a different, more stringent, standard of patentability, solely as a result of recent changes in the Patent Office's application of the requirements of patentability.

### CONCLUSION

For the reasons given above, Appellants submit that claims 33, 38-40, and 44-47 are patentable over Young *et al.* under 35 USC 102(e).

Accordingly, reversal of the rejection of claims 33, 38-40, and 44-47 under 35 § U.S.C. 102(e) as being anticipated by Young *et al.* is respectfully requested.

Please charge any additional fees, including fees for additional extension of time, or credit overpayment to Deposit Account No. 08-1641 (referencing Attorney's Docket No. 39780-2830 P1C53). Please direct any calls in connection with this application to the undersigned at the number provided below.

Respectfully submitted,

Date: August 15, 2005

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8. CLAIMS APPENDIX

Claims on Appeal

33. An isolated nucleic acid comprising:
- (a) the nucleic acid sequence of SEQ ID NO:215;
  - (b) the full-length coding sequence from within the nucleic acid sequence of SEQ ID NO:215; or
  - (c) the full-length coding sequence of the cDNA deposited under ATCC accession number 203226.
38. The isolated nucleic acid of Claim 33 comprising the nucleic acid sequence of SEQ ID NO:215.
39. The isolated nucleic acid of Claim 33 comprising the full-length coding sequence of the nucleic acid sequence of SEQ ID NO:215.
40. The isolated nucleic acid of Claim 33 comprising the full-length coding sequence of the cDNA deposited under ATCC accession number 203226.
44. A vector comprising the nucleic acid of Claim 33.
45. The vector of Claim 44, wherein said nucleic acid is operably linked to control sequences recognized by a host cell transformed with the vector.
46. A host cell comprising the vector of Claim 44.
47. The host cell of Claim 46, wherein said cell is a CHO cell, an *E. coli* or a yeast cell.

## 9. EVIDENCE APPENDIX

1. Young, P.E. et al., U. S. Patent No. 6,444,790, "Peptidoglycan recognition proteins," filed December 22, 1999.

Made of record by the Examiner in the Office Action mailed June 17, 2004.

2. Declaration of David Botstein, Ph.D., Audrey Goddard, Ph.D., Paul J. Godowski, Ph.D., Austin Gurney, Ph.D., Margaret Roy, Colin K. Watanabe, and William I. Wood, Ph.D. under 37 C.F.R. §1.131.

Submitted with Appellants' Supplemental Response filed November 23, 2004, and noted as having been considered by the Examiner in the Office Action mailed December 8, 2004

3. Selsted, M.E, WO 97/29765, "Antimicrobial peptides and methods of use," published August 21, 1997.

Made of record in Appellants' IDS filed September 12, 2002. Initialed as considered by the Examiner on June 8, 2004.

4. Sequence alignment between SEQ ID NO:3 from WO 97/29765 (bovine granulocyte peptide A precursor) and SEQ ID NO:5 (mouse granulocyte peptide A precursor) from WO 97/29765.

5. Selsted, M.E., U.S. Patent No. 6,696,559, "Antimicrobial peptides and methods of use," issued February 24, 2004, filed October 19, 1999.

Item 3 was made of record in Appellants' IDS filed September 12, 2002, and initialed as considered by the Examiner on June 8, 2004. Item 4 is a sequence alignment of two sequences from Item 3, and Item 5 is the U.S. equivalent of Item 3.

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US006444790B1

(12) **United States Patent**  
Young et al.

(10) Patent No.: **US 6,444,790 B1**  
(45) Date of Patent: **Sep. 3, 2002**

(54) **PEPTIDOGLYCAN RECOGNITION PROTEINS**

(75) Inventors: **Paul E. Young, Gaithersburg; Steven M. Ruben, Olney; Craig A. Rosen, Laytonsville; Henrik S. Olsen, Gaithersburg, all of MD (US)**

(73) Assignee: **Human Genome Sciences, Inc., Rockville, MD (US)**

(\*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/469,242**

(22) Filed: **Dec. 22, 1999**

**Related U.S. Application Data**

(60) Provisional application No. 60/113,809, filed on Dec. 23, 1998, now abandoned.

(51) Int. Cl.<sup>7</sup> ..... **C07K 1/00; A61K 38/00; C12P 21/06**

(52) U.S. Cl. .... **530/350; 530/300; 435/69.1**

(58) Field of Search ..... **530/300, 350, 435/69.1**

(56) **References Cited**

**U.S. PATENT DOCUMENTS**

6,034,217 A 3/2000 Ashida et al.

**FOREIGN PATENT DOCUMENTS**

WO	WO97/29765	8/1997
WO	98/51790	11/1998
WO	WO99/02686	1/1999
WO	WO99/58660	11/1999
WO	WO00/39327	7/2000

**OTHER PUBLICATIONS**

Bowie et al (Science, 1990, 257:1306-1310).\*

Burgess et al (J of Cell Bio. 111:2129-2138, 1990).\*

Scott et al (Nature Genetics, 1999, 21:440-443).\*

Bork (Genome Research, 2000, 10:398-400).\*

Reiger et al (Glossary of Genetics and Cytogenetics, Classical and Molecular, 4th Ed., Springer-Verlag, Berlin, 1976).\*

Herbert et al. (The Dictionary of Immunology, Academic Press, 4th edition, 1995).\*

Greenspan et al., (Nature Biotechnology 7:936-937 (1999).\*

Lazar et al. Molecular and Cellular Biology 8:1247-1252, 1988.\*

Labeit et al. (GENBANK™ Sequence Database (Accession Q15346), National Center for Biotechnology Information, National Library of Medicine, Bethesda, Maryland, publicly available Nov., 1996).\*

TrEMBL Accession No. 075594 (Nov. 1, 1998).

Geneseq Database Accession No. Y00771 (Jan. 21, 1999).

GenBank Accession No. AI056693 (Jul. 20, 1998).

Geneseq Database Accession No. Y96963 (Jul. 6, 2000).

Geneseq Database Accession No. Y76124 (Nov. 18, 1999).

Geneseq Database Accession No. Y96962 (Jul. 6, 2000).

Geneseq Database Accession No. A51718 (Jul. 6, 2000).

Geneseq Database Accession No. A51717 (Jul. 6, 2000).

Geneseq Database Accession No. Z65250 (Nov. 18, 1999).

NCBI Accession No. AAF99599 (Aug. 15, 2000).

NCBI Accession No. AF086392 (Aug. 29, 1998).

NCBI Accession No. AAC31822 (Dec. 14, 1998).

Kang et al, "A peptidoglycan recognition protein in innate immunity conserved from insects to humans," Proc. Natl. Acad. Sci. USA, 95:1078-1082 (Aug. 1998).

\* cited by examiner

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(57) **ABSTRACT**

The present invention relates to three novel peptidoglycan recognition binding proteins expressed by keratinocytes, wound-healing tissues and chondrosarcoma tissue. More specifically, isolated nucleic acid molecules are provided encoding human peptidoglycan recognition protein-related proteins, referred to herein as PGRP-K (Keratinocytes), PGRP-W (Wound-healing), and PGRP-C (Chondrosarcoma) of FIGS. 1A-B, FIGS. 2A-C, and FIG. 3, respectively, each having homology to both human peptidoglycan recognition protein (PGRP) as well as murine Tag-7. PGRP-K, PGRP-W, and PGRP-C polypeptides are also provided. Further provided are vectors, host cells and recombinant methods for producing the same. The invention also relates to both the inhibition and enhancement of activities of PGRP-K, PGRP-W, and PGRP-c polypeptides and diagnostic methods for detecting PGRP-K, PGRP-W, and PGRP-C gene expression.

**79 Claims, 11 Drawing Sheets**

Figure 1A

1 CTGGGCTGGACAGCACAGAACCCACAGGGCTGCCGTCCACACTCTCCCGGTGAGAGTCC 60  
61 TGGGACCATGGGGAAGCTGGCATGGCTTCTTGCCCTCTTCATCTGGGTCTCCAGGCT 120  
1 M G T L P W L L A F F I L G L Q A 17  
121 TGGGATACTCCACCATGCTCTCCCGCAAGGAGTGGGGGCAAGACCGCTCGCCTGCAGG 180  
18 W D T P T I V S R K E W G A R P L A C R 37  
181 GCCCTGCTGACCCCTGCCTGTGGCCTACATCATCACAGACCAGCTCCAGGGATGCAGTGC 240  
38 A L L T L P V A Y I I T D Q L P G M Q C 57  
241 CAGCAGCAGAGCGTTTGCAGCCAGATGCTGGGGGGTTCAGTCCCATTCGGTCTACACC 300  
58 Q Q Q S V C S Q M L R G L Q S H S V Y T 77  
301 ATAGGCTGGTGGAGCGTGGCGTACAACTTCTCTGGTGGGGATGATGGCAGGGTGTATGAA 360  
78 I G W C D V A Y N F L V G D D G R V Y E 97  
361 GGTGTGGCTGGAAACATCCAAGGCTTGCACACCCAGGGCTACAACAACATTTCCCTGGGC 420  
98 G V G W N I Q G L H T Q G Y N N I S L G 117  
421 ATCGCCTTCTTTGGCAATAAGATAAGCAGCAGTCCCAGCCCTGCTGCCTTATCAGCTGCA 480  
118 I A F F G N K I S S S P S P A A L S A A 137  
481 GAGGGTCTGATCTCCTATGCCATCCAGAAGGGTCACTGTGGCCAGGTATATTCAGCCA 540  
138 E G L I S Y A I Q K G H L S P R Y I Q P 157  
541 CTTCTTCTGAAAGAAGAGAAGCTGCCCTGGACCCCTCAACATCCAGTGTATGCCAGGAAGGT 600  
158 L L L K E E T C L D P Q H P V M P R K V 177  
601 TGCCCAACATCATCAAAAGATCTGCTTGGGAAGCCAGAGAGACACACTGCCCTAAAATG 660  
178 C P N I I K R S A W E A R E T H C P K M 197  
661 AACCTCCAGCCAAATATGTCATCATCATCCACACCGCTGGCACAAGCTGCAGTGTATCC 720  
198 N L P A K Y V I I I H T A G T S C T V S 217  
721 ACAGACTGCCAGACTGTGGTCCGAAACATACAGTCCCTTTCACATGGACACACGGAACTTT 780  
218 T D C Q T V V R N I Q S F H M D T R N F 237  
781 TGTGACATGGATATCAATAAGGCCAGGCGTGGGGGGATACGTCTGTAAATCCAGGAC 840  
238 C D I G Y Q 243  
841 TTTGGGAGGCCAAGGGGGCAGATCACTTCAGGCCAGGAATTCAAGAGCAGCCTGGCCAA 900

Figure 1B

901 TATGGCGAACTCTGTCTCTACTGAAAACAAACAAACAAACAAACAAAGAAA 960  
961 CAACAAAAATTAGCCGGGTGTGGTGGCACACGCCTGTAGTCCCAGCTACTCAGGAGGCTG 1020  
1021 AGGCATAAGAATTGCTTGAACCCCTGGAGGCGGAGGTTGCAGTGAGCTGAGATTGGGCCAC 1080  
1081 CGCACTCCAGTCTGGGAGACAGAGTGAGACTGTCTCAAAACAAACAAAAAATCCCTA 1140  
1141 ACATAATCTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 1182

Figure 2A

1	GGGCAAGCTGACTGCACCCCTGACCTGCTGGGCTGGGACAGCACAGGACCCACAGATATCT	60
61	GCTGCCATCCACACTCTCCAGATTGGTGTCTCTGGGACCACTGGGGATGCTGCTGTGGCT	120
1		5
	M L L W L	
121	TCTTGTCTTCTCTGCTCTGGGTATCCAGGCCTGGGGTGATTCTCTGGAACAAAACACA	180
6	L V F S A L G I Q A W G D S S W N K T Q	25
181	AGCTAAACAGGTATCAGAGGGGCTCCAGTACCTATTTGAGAACATCTOCCAGCTCACTGA	240
26	A K Q V S E G L Q Y L F E N I S Q L T E	45
241	AAAAGATGTCTOCCACCGGTCTCTCGCAAGGCATGGGGGGCAGAAGCTGTTGGCTGCAG	300
46	K D V S T T V S R K A W G A E A V G C S	65
301	TATTCAGCTGACCAAGCCAGTGAATGTCTTGTATACACCATGTCCCTGGACTGGAGTG	360
66	I Q L T T P V N V L V I H H V P G L E C	85
361	TCAAGACCAGACAGTCTGCAGCCAGAGACTGCGGGAAGTGCAGGCCCATCATGTCCACAA	420
86	H D Q T V C S Q R L R E L Q A H H V H N	105
421	CAACAGTGGGTGTGATGTGGCTTACAACCTTCTGTTGGGGATGATGGCAGGGTGTATGA	480
106	N S G C D V A Y N F L V G D D G R V Y E	125
481	AGGTGTTGGCTGGAATATCCAAGGAGTGCACACCAAGGCTACAACAACATCTCCCTGGG	540
126	G V G W N I Q G V H T Q G Y N N I S L G	145
541	CMTTGCCTTCTTGGGCACTAAGAAAGGOCACAGTCCCAGCCCTGCTGCCCTGTGGCCAT	600
146	F A F F G T K K G H S P S P A A L S A M	165
601	GGAAAACCTAATCACCTATGCTGTCCAGAAGGGCCACCTGTCTATCCAGTTATGTTTCAAGC	660
166	E N L I T Y A V Q K G H L S S S Y V Q P	185
661	ACTTCTTGGGAAGGGCAGAACTGCCTGGCCCCCTGGGCAGAAGACAAGCCTGAAGAAGCT	720
186	L L G K G E N C L A P R Q K T S L K K L	205
721	TGCCCCGGCATGTGCCACGGTCTGTGTGGGGAGCCAGGGAGACCACTGTCCAGGATGAC	780
206	A P A L S H G L C G E P G R P L S R M T	225
781	TCTOCCAGCGAAGTATGGCATATTATCCCACTGCCGGGAGGACCTGCAACATTTCTGA	840
226	L P A K Y G I I I H T A G R T C N I S D	245

Figure 2B

841	TGAGTGGCGCCTGCTGGTCCGGGACATCCAGTCTTTCTACATAGACAGGCTCAAGTCATG	900
246	E C R L L V R D I Q S F Y I D R L K S C	265
901	CGACATTGGTTATAACTTCCTGGTGGGCCAGGATGGCGCCATTTATGAAGGGGTGGGCTG	960
266	D I G Y N F L V G Q D G A I Y E G V G W	285
961	GAATGTCCAAGGCTCCTCCACCCCTGGCTACGATGACATTGCCCTGGGCATTACCTTCAT	1020
286	N V Q G S S T P G Y D D I A L G I T F M	305
1021	GGGCACCTTCACAGGTATACCAACCAATGCTGCAGCACTAGAGGCAGCCCAAGACCTGAT	1080
306	G T F T G I P P N A A A L E A A Q D L I	325
1081	CCAGTGTGCCATGGTCAAAGGGTACCTGACTCCCAACTACCTGCTGGTGGGCCACAGTGA	1140
326	Q C A M V K G Y L T P N Y L L V G H S D	345
1141	TGTGGCCCGAACCTTGTCTCCTGGGCAGGCTTTGTACAACATCATCAGCACCTGGCCTCA	1200
346	V A R T L S P G Q A L Y N I I S T W P H	365
1201	TTTCAAACACTGAGAGAAGCCCCAGGTCTTCTGAGACTGCTTTCCCTCCCTGTCAGGT	1260
366	F K H	368
1261	CTCTCCTGTCTTAACCATCCAGCTTGGCTCAACACCTTTTGCCCTCCTCCCCTGCCACAC	1320
1321	AGTCTGTGCCTCCTTTTTCAGGTTGGGATGATCATGCCTCTCCTGCCAACATCCTOCAC	1380
1381	GGGCTCCAAACTCATAGCTGGACATTCACAGCCCTCTGAGTCTGAGTCCAGATTCTCTC	1440
1441	TCTCCTTACTTCCTCTCCCTTGGAAACCAACTCCTCAGCCAGGTGAGACAATGGGCTGG	1500
1501	TTCTGTGTTTCATTCTCTCTCTCTCCATTCCTCTGCTGGTGAGCCTTCCCTGGT	1560
1561	GTCTGCCTGGCAGCCCCCAACCAACCACTATCACCCCTCACCCATAACTCAGGTCAACGT	1620
1621	GACCAACCTTCCTTGCTTACACATAAACTTGTATATATTTGGATGTAGCCCTTATTTAAT	1680
1681	GGCTGTCAATTATTTATAGATATGTCTATCCTTGCTACTTGGTTGTGAGTTTCTCCAGGG	1740
1741	AGGAACTGTGTTTTATTTCATCTCTATGTCTCTGTTTCTCAGCAGTGTCTGAAATTTAAT	1800
1801	GGGTTCTACTGATGTTTATTAGAGAAATGGATGAATAAATGAATGAAGAGATCCAAAAA	1860

Figure 2C

1861 AAAAAAAAAAAAAAAAAA 1876

Figure 3

1 GATCCCCCGGGCTGCAGGAATTCCGGCAGAGCCCGAACCCCTGCCGOCCTGCCACTATGTCC 60  
1 M S 2

61 CGCCGCTCTATGCTGCTTGCCTGGGCTCTCCCCAGCCTCCTTGGACTCGGAGGGGCTCAG 120  
3 R R S M L L A W A L P S L L R L G A A Q 22

121 GAGACAGAAGACCCGGCTGCTGCAGCCCCATAGTGCCCCGGAACGAGTGGAGGGCCCTG 180  
23 E T E D P A C C S P I V P R N E W K A L 42

181 GCATCAGAGTGGGCCCAGCACCTGAGCCTGCCCCCTACGCTATGTGGTGGTATCGCACACG 240  
43 A S E C A Q H L S L P L R Y V V V S H T 62

241 GCGGGCAGCAGCTGCAACACCCCCGCTCGTGCCAGCAGGCCCCGGAATGTGCAGCAC 300  
63 A G S S C N T P A S C Q Q Q A R N V Q H 82

301 TACCACATGAAGACACTGGGCTGGTGCGACGTGGGCTACAACCTCCTGATTGGAGAAGAC 360  
83 Y H M K T L G W C D V G Y N F L I G E D 102

361 GGGCTCGTATACGAGGGCCGTGGCTGGAACCTCACGGGTGCCCACTCAGGTCACTTATGG 420  
103 G L V Y E G R G W N F T G A H S G H L W 122

421 AACCCCATGTCCATTGGCATCAGCTTCATGGGCAACTACATGGATCGGGTGCCACACCC 480  
123 N P M S I G I S F M G N Y M D R V P T P 142

481 CAGGCCATCCGGGCAGCCAGGGTCTACTGGCCTGGGTGTGGCTCAGGGAGCCCTGAGG 540  
143 Q A I R A A Q G L L A C G V A Q G A L R 162

541 TCCAATATGTGCTCAAAGGACACCGGGATGTGCAGCGTACACTCTCTCCAGGCAACCAG 600  
163 S N Y V L K G H R D V Q R T L S P G N Q 182

601 CTCTACCACCTCATCCAGAATTGGCCACACTACCGCTCCCCCTGAGGCCCTGCTGATCCG 660  
183 L Y H L I Q N W P H Y R S P 196

661 CACCCCATTCCTCCCTCCCATGGCCAAAAACCCCACTGTCTCTCTCCAATAAAGATG 720

721 TAGCTCAAAAAAAAAAAAAAAAAAAAAA 749

Figure 4A

	10	20	30	40	
1	E G T C P W L L A F F	I L G L C	A W		PGRP-K (HKAB265)
1	W - - L L W L L V F S	A I G I Q	A W G D S S W N K T Q		PGRP-W (HWHGB15)
1	W - - S R R S M L L A W A L P S L	I R L G A A Q E T E D P A			PGRP-C (HCDOP40)
1	W - - L F A C A L L A	L E G L A	T		Mouse Tag-7
	50	60	70	80	
19		D T P T I	V S H K E N G A R P L A C R		PGRP-K (HKAB265)
26	A K Q V S E G L Q Y L F E N I S Q L T E K D V S T T	V S R K A W G A E A V G C S			PGRP-W (HWHGB15)
29		C C S P I V F R N E W K A L A S E C A			PGRP-C (HCDOP40)
16		S C S F I V P H S E M R A L P S E C S			Mouse Tag-7
	90	100	110	120	
38	A L I T L E V A Y I I T C Q L P G M Q C Q Q Q S V C S Q M L E G L C S H S V Y T				PGRP-K (HKAB265)
66	I Q L T T E V N V L V I R H V P G L E C H D Q T V C S Q R L E E L C A H H V H N				PGRP-W (HWHGB15)
48	Q H I S L E F L R Y V V V S H T A G S S C N T P A S C Q C Q A R N V C H Y H M K T				PGRP-C (HCDOP40)
35	S R L G H E V R Y V V I S H T A G S F C N S P D S C E C Q A F N V C H Y H K N E				Mouse Tag-7
	130	140	150	160	
78	I G W C D V A Y N E L V G D D G R V Y E G V G W N I Q G L H T Q G Y N N I S L G				PGRP-K (HKAB265)
106	N S G C D V A Y N E L V G D D G R V Y E G V G W N I Q G V H T Q G Y N N I S L G				PGRP-W (HWHGB15)
88	L G W C D V G Y N E L I G E D G L V Y E G R G W N F T G A H S				PGRP-C (HCDOP40)
75	L G W C D V A Y N F L I G E D G H V Y E G R G W N I K G D H T				Mouse Tag-7
	170	180	190	200	
118	I A F F G N K I S S S P S P A A L S A A E G L I S Y A I Q K G H L S P R Y I Q P				PGRP-K (HKAB265)
146	F A F F G T K K G H S P S P A A L S A M E N L I T Y A V Q K G H L S S S Y V Q P				PGRP-W (HWHGB15)
119					PGRP-C (HCDOP40)
106					Mouse Tag-7
	210	220	230	240	
158	L L L K E E T C L D P Q H P V M P R K V C P N I I K		R S A W E A R E T H		PGRP-K (HKAB265)
186	L L G K G E N C L A P R Q K T S L K K L A P A L S H G L C G E P G R P L				PGRP-W (HWHGB15)
119			G H L W N P		PGRP-C (HCDOP40)
106			G P I W N P		Mouse Tag-7
	250	260	270	280	
194	C P K M N L P A K Y V I I I H T A G T S C T V S T D C Q T V V R N I Q S F H M D				PGRP-K (HKAB265)
222	- S R M T L P A K Y G I I I H T A G R T C N I S D E C R L L V R D I Q S F Y I D				PGRP-W (HWHGB15)
125					PGRP-C (HCDOP40)
112					Mouse Tag-7
	290	300	310	320	
234	T R N F C D I G Y				PGRP-K (HKAB265)
261	R L K S C D I G Y N F L V G Q D G A I Y E G V G W N V Q G S S T P G Y D I A L				PGRP-W (HWHGB15)
125					PGRP-C (HCDOP40)
112					Mouse Tag-7

Figure 4B

	330	340	350	360	
243	-----	-----	-----	-----	FCRP-K (HKAB265)
301	GITFMGTFTGIP-----	PNAAALEAAQDLIQCAMVKG			FCRP-W (HWHG815)
128	GISFMGNYMDRV-----	PTPQAIRAAQGLLACGVAQG			FCRP-C (HCDDP40)
115	GITFMGNFMDRVRKAGPPCCPKSSGIWGVSGL-----				Mouse Tag-7
	370	380	390		
243	-----	-----	-----	-----Q	FCRP-K (HKAB265)
333	YLTPNYLLVGHSDVARTLSPGQALYNIISTWPHFKH				FCRP-W (HWHG815)
160	ALRSNYVLKGHRDVQRTLSPGNQLYHLIQNWPHYRSP				FCRP-C (HCDDP40)
147	-----	PEIQL			Mouse Tag-7

'Decoration 'Decoration#1': Shade (with solid black) residues that match the consensus named 'Consensus #1' exactly.

Figure 5

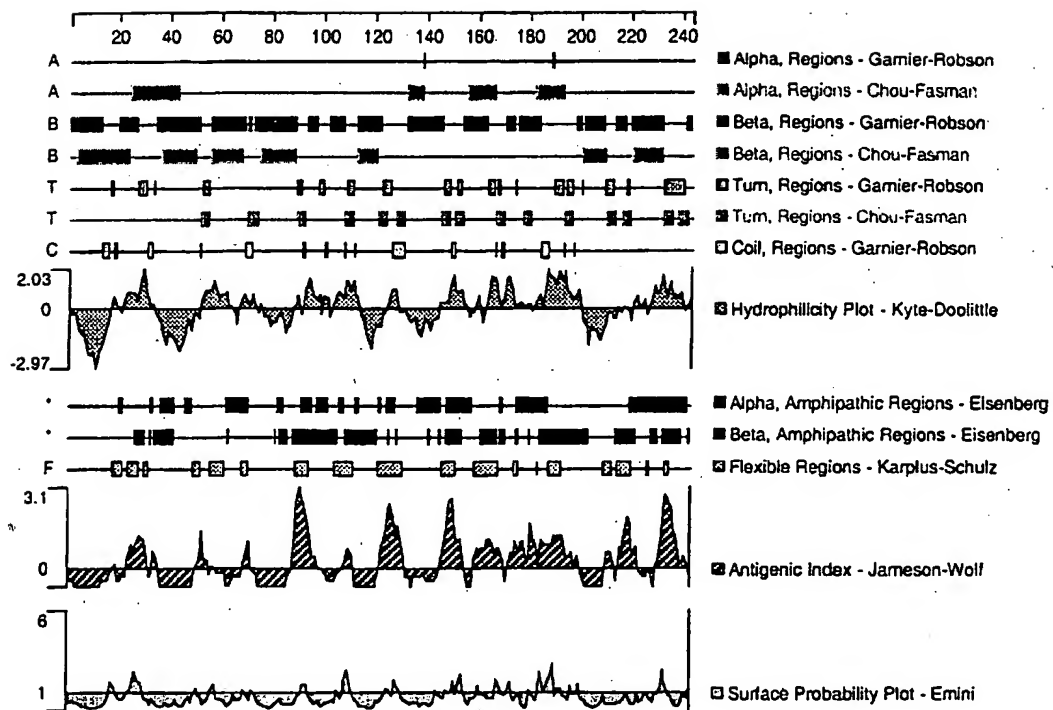


Figure 6

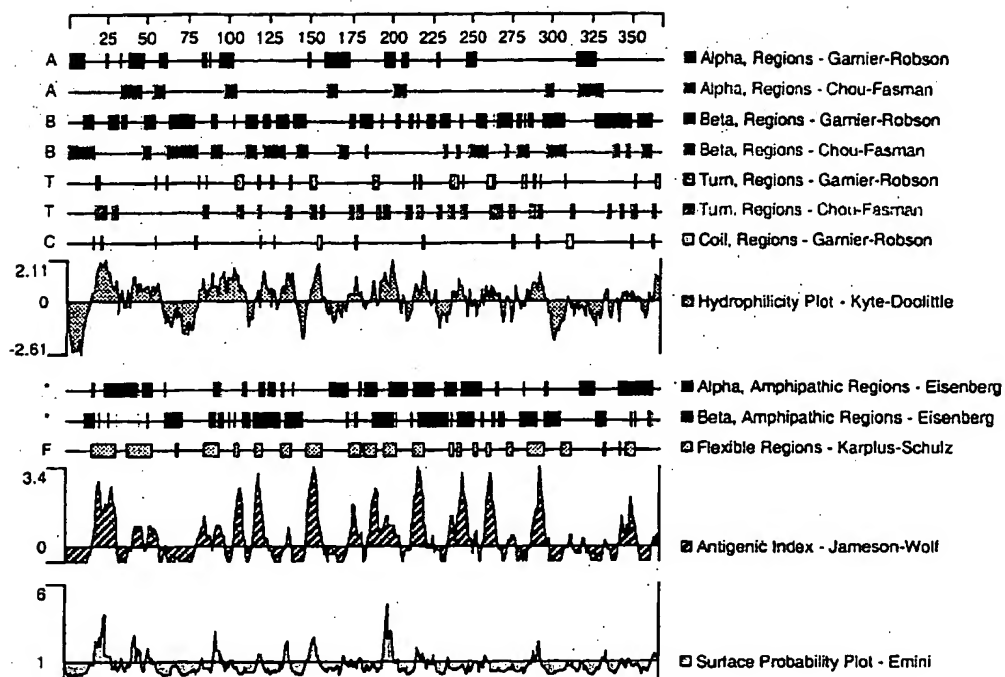
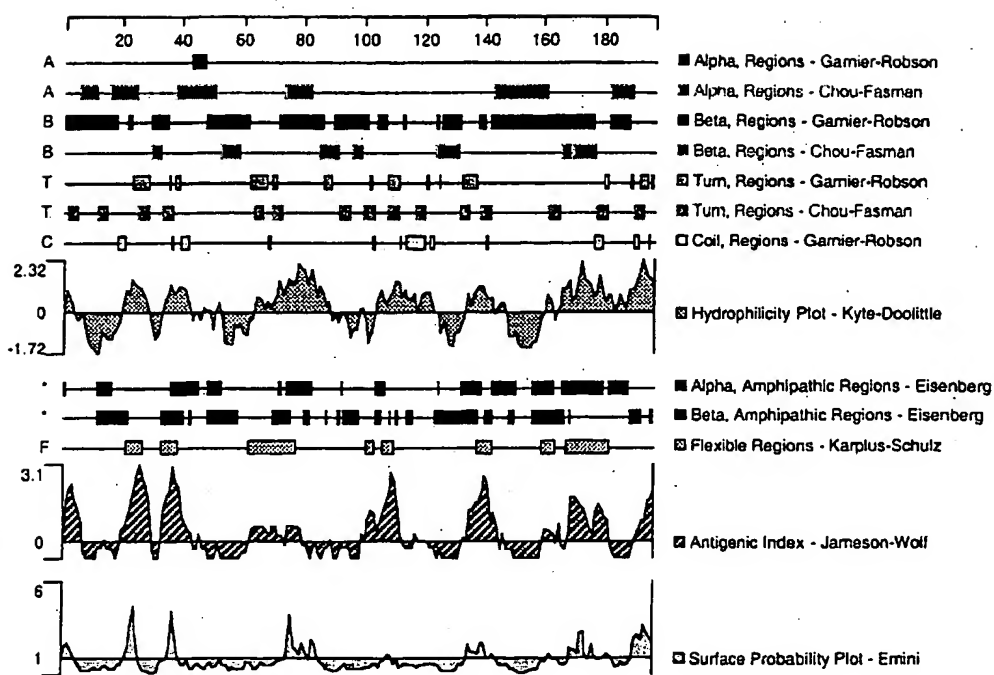


Figure 7



# PEPTIDOGLYCAN RECOGNITION PROTEINS

## FIELD OF THE INVENTION

This application claims benefit under 35 U.S.C. §119(e) of the filing date of U.S. Provisional Application Serial No. 60/113,809, filed on Dec. 23, 1998, now abandoned, which is hereby incorporated by reference.

The present invention relates to three novel peptidoglycan recognition binding proteins expressed by keratinocytes, wound-healing tissues and chondrosarcoma tissue. More specifically, isolated nucleic acid molecules are provided encoding human peptidoglycan recognition protein-related proteins, referred to herein as PGRP-K (Keratinocytes), PGRP-W (Wound-healing), and PGRP-C (Chondrosarcoma) of FIGS. 1A-B, FIGS. 2A-C, and FIG. 3, respectively, each having homology to both human peptidoglycan recognition protein (PGRP) as well as murine Tag-7. PGRP-K, PGRP-W, and PGRP-C polypeptides are also provided. Further provided are vectors, host cells and recombinant methods for producing the same. The invention also relates to both the inhibition and enhancement of activities of PGRP-K, PGRP-W, and PGRP-C polypeptides and diagnostic methods for detecting PGRP-K, PGRP-W, and PGRP-C gene expression.

## BACKGROUND OF THE INVENTION

Peptidoglycan, as well as Lipopolysaccharide (LPS), is a surface component of many bacteria which illicit a wide range of physiological and immune responses in humans. Specifically, peptidoglycan has been shown to manifest itself clinically by reproducing most of the symptoms of bacterial infection, including fever, acute-phase response, inflammation, septic shock, leukocytosis, sleepiness, malaise, abscess formation, and arthritis (see Dziarski et al., JBC, 273 (15): 8680 (1998)). Furthermore, the type of peptidoglycan (i.e.—the specific stereoisomers or analogs of muramyl dipeptide, N-acetylglucosaminyl-beta(1-4)-N-acetylmuramyl tetrapeptides, etc.), were shown to elicit a broad range of activities, including exhibiting greater pyrogenicity, inducing acute joint inflammation, stimulating macrophages, and causing hemorrhagic necrosis at a primed site (See Kotani et al., Fed Proc, 45(11): 2534 (1986)).

It has been demonstrated in humans that a lipopolysaccharide binding protein exists that was discovered as a trace plasma protein (See Schumann et al., Science, 249(4975): 1429 (1990)). It is thought that one of the modes of action by which this lipopolysaccharide binding protein functions is by forming high-affinity complexes with lipopolysaccharide, that then bind to macrophages and monocytes, inducing the secretion of tumor necrosis factor. Dziarski and Gupta (See Dziarski et al., JBC, 269(3): 2100 (1994)) demonstrated that a 70 kDa receptor protein present on the surface of mouse lymphocytes served to bind heparin, heparinoids, bacterial lipoteichoic acids, peptidoglycan, and lipopolysaccharides.

Recently, Dziarski et al. demonstrated that the CD14, a glycosylphosphatidylinositol-linked protein present on the surface of macrophage and polymorphonuclear leukocytes, bound peptidoglycan and lipopolysaccharide. Furthermore, the binding affinity of CD14 for lipopolysaccharide was significantly increased in the presence of a LPS-binding protein present in plasma. It is thought that the LPS-binding protein functions as a transfer molecule, whereby it binds LPS and presents it to the CD14 receptor (See Dziarski et al., JBC, 273(15): 8680 (1998)).

Yoshida et al. isolated a peptidoglycan binding protein from the hemolymph of the Silkworm, *Bombyx mori*, using column chromatography. This protein was found to have a very specific affinity for peptidoglycan (See Yoshida et al., JBC, 271(23): 13854 (1996)). Additionally, Kang et al. recently cloned a peptidoglycan binding protein from the moth *Trichoplusia ni*. The peptidoglycan binding protein was shown to bind strongly to insoluble peptidoglycan (See Kang et al., PNAS, 95(17): 10078 (1998)). In this study the peptidoglycan binding protein was upregulated by a bacterial infection in *T. ni*. The insect immune system is regarded as a model for innate immunity. Thus, Kang et al. were able to clone both mouse and human homologs of the *T. ni* peptidoglycan binding protein. All of these peptidoglycan binding proteins shared regions of homology, as well as four conserved cysteine residues which may function in the tertiary structure of the protein, possibly in helping to form binding domains. Given that peptidoglycan is an integral component of bacterial cell walls, and that it induces many physiological responses from cytokine secretion to inflammation and macrophage activation, it appears as if this family of proteins may be a ubiquitous group involved in the binding and recognition of peptidoglycan, the presentation of antigens (e.g., cell wall components, etc.), and the activation of the immune system, such as the secretion of cytokines, such as TNF.

TNF is noted for its pro-inflammatory actions which result in tissue injury, such as induction of procoagulant activity on vascular endothelial cells (Pober, J. S. et al., *J. Immunol.* 136:1680 (1986)), increased adherence of neutrophils and lymphocytes (Pober, J. S. et al., *J. Immunol.* 138:3319 (1987)), and stimulation of the release of platelet activating factor from macrophages, neutrophils and vascular endothelial cells (Camussi, G. et al., *J. Exp. Med.* 166:1390 (1987)).

Recent evidence implicates TNF in the pathogenesis of many infections (Cerami, A. et al., *Immunol. Today* 9:28 (1988)), immune disorders, neoplastic pathology, e.g., in cachexia accompanying some malignancies (Oliff, A. et al., *Cell* 50:555 (1987)), and in autoimmune pathologies and graft-versus host pathology (Piguet, P.-F. et al., *J. Exp. Med.* 166:1280 (1987)). The association of TNF with cancer and infectious pathologies is often related to the host's catabolic state. A major problem in cancer patients is weight loss, usually associated with anorexia. The extensive wasting which results is known as "cachexia" (Kern, K. A. et al. *J. Parent. Enter. Nutr.* 12:286-298 (1988)). Cachexia includes progressive weight loss, anorexia, and persistent erosion of body mass in response to a malignant growth. The cachectic state is thus associated with significant morbidity and is responsible for the majority of cancer mortality. A number of studies have suggested that TNF is an important mediator of the cachexia in cancer, infectious pathology, and in other catabolic states.

TNF is thought to play a central role in the pathophysiological consequences of Gram-negative sepsis and endotoxin shock (Michie, H. R. et al., *Br. J. Surg.* 76:670-671 (1989); Debets, J. M. H. et al., *Second Vienna Shock Forum*, p.463-466 (1989); Simpson, S. Q. et al., *Crit. Care Clin.* 5:27-47 (1989)), including fever, malaise, anorexia, and cachexia. Endotoxin is a potent monocyte/macrophage activator which stimulates production and secretion of TNF (Kombuth, S. K. et al., *J. Immunol.* 137:2585-2591 (1986)) and other cytokines. Because TNF could mimic many biological effects of endotoxin, it was concluded to be a central mediator responsible for the clinical manifestations of endotoxin-related illness. TNF and other monocyte-derived

cytokines mediate the metabolic and neurohormonal responses to endotoxin (Michie, H. R. et al., *N. Eng. J. Med.* 318:1481-1486 (1988)). Endotoxin administration to human volunteers produces acute illness with flu-like symptoms including fever, tachycardia, increased metabolic rate and stress hormone release (Revhaug, A. et al., *Arch. Surg.* 123:162-170 (1988)). Elevated levels of circulating TNF have also been found in patients suffering from Gram-negative sepsis (Waage, A. et al., *Lancet* 1:355-357 (1987); Hammerle, A. F. et al., *Second Vienna Shock Forum* p. 715-718 (1989); Debets, J. M. H. et al., *Crit. Care Med.* 17:489-497 (1989); Calandra, T. et al., *J. Infect. Dis.* 161:982-987 (1990)).

Passive immunotherapy directed at neutralizing TNF may have a beneficial effect in Gram-negative sepsis and endotoxemia, based on the increased TNF production and elevated TNF levels in these pathology states, as discussed above. Antibodies to a "modulator" material which was characterized as cachectin (later found to be identical to TNF) were disclosed by Cerami et al. (EPO Patent Publication 0,212,489, Mar. 4, 1987). Such antibodies were said to be useful in diagnostic immunoassays and in therapy of shock in bacterial infections. Rubin et al. (EPO Patent Publication 0,218,868, Apr. 22, 1987) disclosed monoclonal antibodies to human TNF, the hybridomas secreting such antibodies, methods of producing such antibodies, and the use of such antibodies in immunoassay of TNF. Yone et al. (EPO Patent Publication 0,288,088, Oct. 26, 1988) disclosed anti-TNF antibodies, including mAbs, and their utility in immunoassay diagnosis of pathologies, in particular Kawasaki's pathology and bacterial infection. The body fluids of patients with Kawasaki's pathology (infantile acute febrile mucocutaneous lymph node syndrome; Kawasaki, T., *Allergy* 16:178 (1967); Kawasaki, T., *Shonika (Pediatrics)* 26:935 (1985)) were said to contain elevated TNF levels which were related to progress of the pathology (Yone et al., *supra*).

Accordingly, there is a need to provide molecules that are involved in pathological conditions. Such novel proteins could be useful in augmenting the immune system in such areas as immune recognition, antigen presentation, and immune system activation. Antibodies or antagonists directed against these proteins may be useful in reducing or eliminating disorders associated with TNF and TNF-like cytokines, such as endotoxic shock and auto-immune disorders, for example.

### SUMMARY OF THE INVENTION

The present invention provides isolated nucleic acid molecules comprising polynucleotides encoding three novel proteins that are structurally similar to a human Peptidoglycan Recognition Protein and murine Tag-7, and are believed to have similar biological effects and activities. The cytokines are named PGRP-K, PGRP-W, and PGRP-C, and the invention includes PGRP-K, PGRP-W, and PGRP-C polypeptides having at least a portion of the amino acid sequence in FIGS. 1A-B (SEQ ID NO:2), in FIGS. 2A-C (SEQ ID NO:4), and/or FIG. 3 (SEQ ID NO:6) or amino acid sequence encoded by the cDNA clones deposited on Dec. 23, 1998, assigned ATCC number 203564; Dec. 23, 1998, assigned ATCC number 203563; and Mar. 20, 1998, assigned ATCC number 209683, respectively. The nucleotide sequence determined by sequencing the deposited PGRP-K clone, which is shown in FIGS. 1A-B (SEQ ID NO:1), contains an open reading frame encoding a complete polypeptide of 243 amino acid residues including an N-terminal methionine, a predicted PGRP-like domain of

about 83 amino acid residues, and a deduced molecular weight for the complete protein of about 27 kDa.

The nucleotide sequence determined by sequencing the deposited PGRP-W clone, which is shown in FIGS. 2A-C (SEQ ID NO:3), contains an open reading frame encoding a complete polypeptide of 368 amino acid residues including an N-terminal methionine; a predicted PGRP-like domain of about 83 amino acid residues, and a deduced molecular weight for the complete protein of about 40 kDa.

The nucleotide sequence determined by sequencing the deposited PGRP-C clone, which is shown in FIG. 3 (SEQ ID NO:5), contains an open reading frame encoding a complete polypeptide of 196 amino acid residues including an N-terminal methionine, a predicted PGRP-like domain of about 83 amino acid residues, and a deduced molecular weight for the complete protein of about 21 kDa.

Thus, one aspect of the invention provides isolated nucleic acid molecules comprising polynucleotides having nucleotide sequences selected from the group consisting of: (a) a nucleotide sequence encoding a full-length PGRP-K, PGRP-W, or PGRP-C polypeptide having the complete amino acid sequence in FIGS. 1A-B (SEQ ID NO:2), in FIGS. 2A-C (SEQ ID NO:4), or in FIG. 3 (SEQ ID NO:6), respectively, or as encoded by the cDNA clones contained in the ATCC Deposit number 203564, deposited on Dec. 23, 1998; ATCC Deposit number 203563, deposited on Dec. 23, 1998; and ATCC Deposit number 209683, deposited on Mar. 20, 1998, respectively.; (b) a nucleotide sequence encoding the predicted PGRP-like domain of the PGRP-K polypeptide having the amino acid sequence at positions 24 to 107 in FIGS. 1A-B (SEQ ID NO:2), the predicted PGRP-like domain of the PGRP-W polypeptide having the amino acid sequence at positions 52 to 135 in FIGS. 2A-C (SEQ ID NO:4), and/or the predicted PGRP-like domain of the PGRP-C polypeptide having the amino acid sequence at positions 34 to 117 in FIG. 3 (SEQ ID NO:6), or as encoded by the cDNA clones contained in ATCC Numbers 203564, 203563, and 209683, respectively, deposited on Dec. 23, 1998, and Mar. 20, 1998; or as encoded by the cDNA clones contained in ATCC Numbers 203564, 203563, and 209683, respectively, deposited on Dec. 23, 1998, and Mar. 20, 1998; (c) a nucleotide sequence encoding a soluble PGRP-K, PGRP-W, and/or PGRP-C polypeptide having the PGRP-like domain but lacking the leader sequence; and (d) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), or (c) above.

Further embodiments of the invention include isolated nucleic acid molecules that comprise a polynucleotide having a nucleotide sequence at least 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical, to any of the nucleotide sequences in (a), (b), (c), or (d) above, or a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide in (a), (b), (c), or (d) above. This polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues. An additional nucleic acid embodiment of the invention relates to an isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of a PGRP-K, a PGRP-W, or a PGRP-C polypeptide having an amino acid sequence in (a), (b), or above.

The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, and to host cells containing the recombinant vectors, as well as to methods of making such vectors

and host cells and for using them for the production of PGRP-K, PGRP-W, and/or PGRP-C polypeptides or peptides by recombinant techniques.

The invention further provides isolated PGRP-K, PGRP-W, and PGRP-C polypeptides comprising amino acid sequences selected from the group consisting of: (a) the amino acid sequence of the full-length PGRP-K polypeptide having the complete amino acid sequence shown in FIGS. 1A-B (SEQ ID NO:2), the amino acid sequence of the full-length PGRP-W polypeptide having the complete amino acid sequence shown in FIGS. 2A-C (SEQ ID NO:4), the amino acid sequence of the full-length PGRP-C polypeptide having the complete amino acid sequence shown in FIG. 3 (SEQ ID NO:6), or as encoded by the cDNA clones contained in ATCC Numbers 203564, 203563, and 209683, respectively, deposited on Dec. 23, 1998, and Mar. 20, 1998; (b) the amino acid sequence of the predicted PGRP-like domain of the PGRP-K polypeptide having the amino acid sequence at positions 24 to 107 in FIGS. 1A-B (SEQ ID NO:2), the predicted PGRP-like domain of the PGRP-W polypeptide having the amino acid sequence at positions 52 to 135 in FIGS. 2A-C (SEQ ID NO:4), and/or the predicted PGRP-like domain of the PGRP-C polypeptide having the amino acid sequence at positions 34 to 117 in FIG. 3 (SEQ ID NO:6), or as encoded by the cDNA clones contained in ATCC Numbers 203564, 203563, and 209683, respectively, deposited on Dec. 23, 1998, and Mar. 20, 1998; (c) the amino acid sequence of the soluble PGRP-K, PGRP-W, and/or PGRP-C polypeptide having the PGRP-like domain but lacking the leader sequence, wherein each of these domains is defined below.

The polypeptides of the present invention also include polypeptides having an amino acid sequence with at least 90% similarity, and more preferably at least 95% similarity to those described in (a), (b), or (c) above, as well as polypeptides having an amino acid sequence at least 80% identical, more preferably at least 90% identical, and still more preferably 95%, 96%, 97%, 98% or 99% identical to those above.

An additional embodiment of this aspect of the invention relates to a peptide or polypeptide which has the amino acid sequence of an epitope-bearing portion of a PGRP-K, a PGRP-W, or a PGRP-C polypeptide having an amino acid sequence described in (a), (b), or (c) above. Peptides or polypeptides having the amino acid sequence of an epitope-bearing portion of a PGRP-K, a PGRP-W, or a PGRP-C polypeptide of the invention include portions of such polypeptides with at least six or seven, preferably at least nine, and more preferably at least about 30 amino acids to about 50 amino acids, although epitope-bearing polypeptides of any length up to and including the entire amino acid sequence of a polypeptide of the invention described above also are included in the invention. In another embodiment, the invention provides an isolated antibody that binds specifically to a polypeptide having an amino acid sequence described in (a), (b), or (c) above.

The invention further provides methods for isolating antibodies that bind specifically to an PGRP-K, PGRP-W, or PGRP-C polypeptide having an amino acid sequence as described herein. Such antibodies are useful diagnostically or therapeutically as described below.

The invention also provides for pharmaceutical compositions comprising soluble PGRP-K, PGRP-W, and/or PGRP-C polypeptides, particularly human PGRP-K, PGRP-W, and/or PGRP-C polypeptides, which may be employed, for instance, to treat tumor and tumor metastasis, infections

by bacteria, viruses and other parasites, immunodeficiencies, inflammatory diseases, regulate the apoptosis and/or proliferation of keratinocytes, epidermal cells, and epithelial cells, mediate antigen processing and presentation, mediate cell activation and proliferation, and are functionally linked as primary mediators of immune recognition and immune responses.

The invention further provides compositions comprising a PGRP-K, PGRP-W, or PGRP-C polynucleotide or a PGRP-K, PGRP-W, or PGRP-C polypeptide for administration to cells in vitro, to cells ex vivo and to cells in vivo, or to a multicellular organism. In certain particularly preferred embodiments of this aspect of the invention, the compositions comprise a PGRP-K, PGRP-W, or PGRP-C polynucleotide for expression of a PGRP-K, PGRP-W, or PGRP-C polypeptide in a host organism for treatment of disease. Particularly preferred in this regard is expression in a human patient for treatment of a dysfunction associated with aberrant endogenous activity of a PGRP-K, PGRP-W, or PGRP-C gene.

The present invention also provides a screening method for identifying compounds capable of enhancing or inhibiting a cellular response induced by PGRP-K, PGRP-W, or PGRP-C which involves contacting cells which express PGRP-K, PGRP-W, or PGRP-C with the candidate compound, assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made in absence of the candidate compound; whereby, an increased cellular response over the standard indicates that the compound is an agonist and a decreased cellular response over the standard indicates that the compound is an antagonist.

In another aspect, a method for identifying PGRP-K, PGRP-W, or PGRP-C receptors is provided, as well as a screening assay for agonists and antagonists using such receptors. This assay involves determining the effect a candidate compound has on PGRP-K, PGRP-W, or PGRP-C binding to the PGRP-K, PGRP-W, or PGRP-C receptor. In particular, the method involves contacting a PGRP-K, PGRP-W, or PGRP-C receptor with an PGRP-K, PGRP-W, or PGRP-C polypeptide and a candidate compound and determining whether PGRP-K, PGRP-W, or PGRP-C polypeptide binding to the PGRP-K, PGRP-W, or PGRP-C receptor is increased or decreased due to the presence of the candidate compound. The antagonists may be employed to prevent septic shock, inflammation, and to regulate the growth activity of keratinocytes.

The present inventors have discovered that PGRP-K, PGRP-W, and PGRP-C is expressed in keratinocytes, wound healing tissues, and chondrosarcomas, respectively. For a number of disorders of these tissues and cells, such as tumor and tumor metastasis, infection of bacteria, viruses and other parasites, immunodeficiencies, septic shock, apoptosis or proliferation of these tissues, and proper antigen processing and presentation, it is believed that significantly higher or lower levels of the PGRP-K, PGRP-W, or PGRP-C gene expression can be detected in certain tissues (e.g., keratinocytes, wound-healing tissues, and chondrosarcoma) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" PGRP-K, PGRP-W, or PGRP-C gene expression level, i.e., the PGRP-K, PGRP-W, or PGRP-C expression level in tissue or bodily fluids from an individual not having the disorder. Thus, the invention provides a diagnostic method useful during diagnosis of a disorder, which involves: (a) assaying PGRP-K, PGRP-W, or PGRP-C gene expression levels in cells or body fluid of

an individual; (b) comparing the PGRP-K, PGRP-W, or PGRP-C gene expression level with a standard PGRP-K, PGRP-W, or PGRP-C gene expression level, whereby an increase or decrease in the assayed PGRP-K, PGRP-W, or PGRP-C gene expression level compared to the standard expression level is indicative of a disorder.

An additional aspect of the invention is related to a method for treating an individual in need of an increased level of either PGRP-K, PGRP-W, or PGRP-C activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an isolated PGRP-K, PGRP-W, or PGRP-C polypeptide of the invention or an agonist thereof.

A still further aspect of the invention is related to a method for treating an individual in need of a decreased level of either PGRP-K, PGRP-W, or PGRP-C activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of a PGRP-K, PGRP-W, or PGRP-C antagonist. Preferred antagonists for use in the present invention are either PGRP-K, PGRP-W, or PGRP-C-specific antibodies.

#### BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1A-B show the nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of the PGRP-K. The deduced complete amino acid sequence includes 243 amino acid residues and has a deduced molecular weight of about 27,000 Da. The predicted domains of the PGRP-K are: signal sequence (amino acid residues Met-1 to about Ala-17 of SEQ ID NO:2) and PGRP-like domain (amino acid residues from about Val-24 to about His-107 of SEQ ID NO:2).

FIGS. 2A-C show the nucleotide sequence (SEQ ID NO:3) and deduced amino acid sequence (SEQ ID NO:4) of the PGRP-W. The deduced complete amino acid sequence includes 368 amino acid residues and has a deduced molecular weight of about 40,286 Da. The predicted domains of the PGRP-W are: signal sequence (amino acid residues Met-1 to about Gly-17 of SEQ ID NO:4) and PGRP-like domain (amino acid residues from about Val-52 to about His-135 of SEQ ID NO:4).

FIGS. 3 show the nucleotide sequence (SEQ ID NO:5) and deduced amino acid sequence (SEQ ID NO:6) of the PGRP-C. The deduced complete amino acid sequence includes 196 amino acid residues and has a deduced molecular weight of about 21,500 Da. The predicted domains of the PGRP-C are: signal sequence (amino acid residues Met-1 to about Ala-21 of SEQ ID NO:6) and PGRP-like domain (amino acid residues from about Val-34 to about His-117 of SEQ ID NO:6).

FIGS. 4A-B show the regions of similarity between the amino acid sequences of the PGRP-K protein of FIGS. 1A-B (labeled PGRP-K (HKABZ65); SEQ ID NO:2), the PGRP-W protein of FIGS. 2A-C (labeled PGRP-W (HWHGB15); SEQ ID NO:4), the PGRP-C protein of FIG. 3 (labeled PGRP-C (HCDDP40); SEQ ID NO:6), and the Mouse Tag-7 protein (SEQ ID NO:7) (GenBank Accession Number X86374), as determined by the "Megalign" routine which is part of the computer program called "DNASTar". Identical amino acid residues between these protein sequences are shaded.

FIG. 5 and Table II show a structural analysis of the PGRP-K amino acid sequence of FIGS. 1A-B (SEQ ID NO:2), generated using the default parameters of the recited computer programs. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions;

flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index—Jameson-Wolf" graph, amino acid residues: Val-24 to Ala-35; from Gln-51 to Gln-58; from Gly-69 to Ser-72; from Leu-88 to Gly-100; from His-107 to Tyr-111; from Gly-122 to Pro-131; from Gln-146 to Ile-155; from Leu-159 to His-170; from Val-172 to Pro-200; from Gly-211 to Val-223; and from Phe-230 to Tyr-242 as depicted in FIGS. 1A-B (SEQ ID NO:2) correspond to the shown highly antigenic regions of the PGRP-K protein.

FIG. 6 and Table III show a structural analysis of the PGRP-W amino acid sequence of FIGS. 2A-C (SEQ ID NO:4), generated using the default parameters of the recited computer programs. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index—Jameson-Wolf" graph, amino acid residues: Gly-17 to Gly-32; from Ile-40 to Gly-58; from Gly-82 to Gln-99; from His-104 to Val-111; from Leu-116 to Glu-125; from Gly-150 to Pro-159; from Gln-174 to Tyr-182; from Leu-186 to Pro-207; from Val-214 to Met-225; from Thr-237 to Val-252; from Tyr-259 to Ile-268; from Gly-290 to Ala-300; from His-344 to Gln-355; and from Trp-364 to His-368 as depicted in FIGS. 2A-C (SEQ ID NO:4) correspond to the shown highly antigenic regions of the PGRP-W protein.

FIG. 7 and Table IV show a structural analysis of the PGRP-C amino acid sequence of FIG. 3 (SEQ ID NO:6), generated using the default parameters of the recited computer programs. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index—Jameson-Wolf" graph, amino acid residues: Met-1 to Met-6; from Ala-20 to Cys-29; from Ile-33 to Ala-43; from Ala-63 to Asn-79; from Ile-99 to Asn-112; from Gly-133 to Arg-146; from Ala-160 to Tyr-165; from Lys-168 to Asn-181; and from Trp-190 to Pro-196 as depicted in FIG. 3 (SEQ ID NO:6) correspond to the shown highly antigenic regions of the PGRP-C protein.

#### DETAILED DESCRIPTION

The present invention provides isolated nucleic acid molecules comprising polynucleotides encoding a PGRP-K, a PGRP-W and a PGRP-C polypeptides (FIGS. 1A-B, 2A-C, and 3 (SEQ ID NO:1, SEQ ID NO:3, and SEQ ID NO:5, respectively), the amino acid sequences of which were determined by sequencing cloned cDNAs. The PGRP-K, PGRP-W, and PGRP-C proteins shown in FIGS. 1A-B, 2A-C, and 3, respectively, share sequence homology with the murine Tag-7 protein (FIGS. 4A-B (SEQ ID NO:7)). On Dec. 23, 1998, and Mar. 20, 1998, deposits of plasmid DNAs encoding PGRP-K, PGRP-W, and PGRP-C were made at the American Type Culture Collection, 10801 University Boulevard, Manassas, Va. 20110-2209, and given accession numbers 203564, 203563, and 209683, respectively. The nucleotide sequences shown in FIGS. 1, 2, and 3 (SEQ ID NO:1, SEQ ID NO:3, and SEQ ID NO:5, respectively) were obtained by sequencing cDNA clones (Clone ID HKABZ65, HWHGB15, and HCDDP40, respectively) containing the same amino acid coding sequences as the clones in ATCC Accession Nos. 203564, 203563, and 209683, respectively. The deposited clone encoding PGRP-K is contained in the pCMVSPORT2.0 plasmid (Life Technologies, Rockville, Md.), the deposited clone encoding PGRP-W is contained in the pCMVSPORT3.0 plasmid (Life Technologies, Rockville, Md.), and the deposited clone encoding the PGRP-C is contained in the Uni-Zap XR plasmid (Stratagene, La Jolla, Calif.).

## Nucleic Acid Molecules

Unless otherwise indicated, all nucleotide sequences determined by sequencing a DNA molecule herein were determined using an automated DNA sequencer (such as the Model 373 from Applied Biosystems, Inc., Foster City, Calif.), and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted by translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by this automated approach, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by automation are typically at least about 90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. The actual sequence can be more precisely determined by other approaches including manual DNA sequencing methods well known in the art. As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

By "nucleotide sequence" of a nucleic acid molecule or polynucleotide is intended, for a DNA molecule or polynucleotide, a sequence of deoxyribonucleotides, and for an RNA molecule or polynucleotide, the corresponding sequence of ribonucleotides (A, G, C and U), where each thymidine deoxyribonucleotide (T) in the specified deoxyribonucleotide sequence is replaced by the ribonucleotide uridine (U).

Using the information provided herein, such as the nucleotide sequences in FIGS. 1 or 2, a nucleic acid molecule of the present invention encoding a peptidoglycan recognition protein polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Illustrative of the invention, the nucleic acid molecule described in FIG. 1 (SEQ ID NO:1) was discovered in a cDNA library derived from Human keratinocytes, the nucleic acid molecule described in FIG. 2 (SEQ ID NO:3) was discovered in cDNA libraries derived from Human keratinocytes and Human tissues undergoing wound-healing, and the nucleic acid molecule described in FIG. 3 (SEQ ID NO:5) was discovered in cDNA libraries derived from Human chondrosarcoma.

The PGRP-K gene contains an open reading frame encoding a protein of about 243 amino acid residues, a PGRP-like domain of about 137 amino acids (amino acid residues from about 18 to about 155 in FIGS. 1A-B (SEQ ID NO:2)), and a deduced molecular weight of about 27 kDa. The PGRP-K protein shown in FIGS. 1A-B (SEQ ID NO:2) is about 40% similar to the mouse Tag-7 protein which can be accessed on Genbank as Accession No. X86374.

The PGRP-W gene contains an open reading frame encoding a protein of about 368 amino acid residues, a PGRP-like domain of about 175 amino acids (amino acid residues from about 18 to about 193 in FIGS. 2A-C (SEQ ID NO:4)), and a deduced molecular weight of about 40.2 kDa. The PGRP-W protein shown in FIGS. 2A-C (SEQ ID NO:4) is about 32% similar to the mouse Tag-7 protein which can be accessed on Genbank as Accession No. X86374.

The PGRP-C gene contains an open reading frame encoding a protein of about 196 amino acid residues, a PGRP-like

domain of about 98 amino acids (amino acid residues from about 23 to about 120 in FIG. 3 (SEQ ID NO:6)), and a deduced molecular weight of about 21.5 kDa. The PGRP-C protein shown in FIG. 3 (SEQ ID NO:6) is about 67% similar to the mouse Tag-7 protein which can be accessed on Genbank as Accession No. X86374.

Furthermore, PGRP-W is 59% homologous to PGRP-K, PGRP-W is 42% homologous to PGRP-C, and PGRP-K is 39% homologous to PGRP-C. More importantly, PGRP-K, PGRP-W, and PGRP-C share a conserved region of homology amongst the sequences, as well as with the murine Tag-7 protein. Also, four conserved cysteines are also homologous amongst all of these sequences, as can be seen in FIGS. 4A-B. Based upon alignments and sequence distances, it appears as if these three PGRPs constitute a novel class of peptidoglycan binding proteins, which share a conserved domain (PGRP-like domain), which may function in binding, while also having significantly diverging regions of homology as well. Thus, it appears as each of these PGRPs is a novel member of a family of peptidoglycan binding proteins thought to play an important role in immune recognition, immune surveillance, antigen presentation, and immune system activation.

As indicated, nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. Single-stranded DNA or RNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include in vivo or in vitro RNA transcripts of the DNA molecules of the present invention. Isolated nucleic acid molecules according to the present invention further include such molecules produced synthetically. Isolated nucleic acid molecules of the present invention include DNA molecules comprising an open reading frame (ORF) with an initiation codon starting at position 70 of the nucleotide sequence shown in FIGS. 1A-B (SEQ ID NO:1), an initiation codon starting at position 106 of the nucleotide sequence shown in FIGS. 2A-C (SEQ ID NO:3), and an initiation codon starting at position 55 of the nucleotide sequence shown in FIG. 3 (SEQ ID NO:5). As one of ordinary skill would appreciate, due to the possibilities of sequencing errors discussed above, the actual complete PGRP-C polypeptide encoded by the deposited cDNA, which comprises about 196 amino acids, may be somewhat shorter. In particular, the determined PGRP-C coding sequence contains a second methionine codon which may serve as an alternative start codon for translation of the open reading frame, at nucleotide positions 70-72 in FIG. 3 (SEQ ID NO:5). More generally, the actual open reading frame may be anywhere in the range of  $\pm 10$  amino acids, more likely in the range of  $\pm 6$  amino acids, of that predicted from either the first or second methionine codon from the N-terminus shown in FIG. 3 (SEQ ID NO:5). In addition, isolated nucleic acid molecules of the invention include DNA molecules which comprise a sequence substantially different from those described above but which, due to the

degeneracy of the genetic code, still encode either the PGRP-K, PGRP-W, or PGRP-C proteins. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate the degenerate variants described above. In another aspect, the invention provides isolated nucleic acid molecules encoding the PGRP-K, PGRP-W, and PGRP-C polypeptides having amino acid sequences encoded by the cDNAs contained in the plasmids deposited on Dec. 23, 1998, and Mar. 20, 1998.

The invention further provides an isolated nucleic acid molecule having the nucleotide sequence shown in FIGS. 1A-B (SEQ ID NO:1) or the nucleotide sequence of the PGRP-K cDNA contained in the above-described deposited clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, are useful as probes for gene mapping, by in situ hybridization with chromosomes, and for detecting expression of the PGRP-K gene in human tissue, for instance, by Northern blot analysis.

The invention further provides an isolated nucleic acid molecule having the nucleotide sequence shown in FIGS. 2A-C (SEQ ID NO:3) or the nucleotide sequence of the PGRP-W cDNA contained in the above-described deposited clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, are useful as probes for gene mapping, by in situ hybridization with chromosomes, and for detecting expression of the PGRP-W gene in human tissue, for instance, by Northern blot analysis.

The invention further provides an isolated nucleic acid molecule having the nucleotide sequence shown in FIG. 3 (SEQ ID NO:5) or the nucleotide sequence of the PGRP-C cDNA contained in the above-described deposited clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, are useful as probes for gene mapping, by in situ hybridization with chromosomes, and for detecting expression of the PGRP-C gene in human tissue, for instance, by Northern blot analysis.

The present invention is further directed to nucleic acid molecules encoding portions of the nucleotide sequences described herein as well as to fragments of the isolated nucleic acid molecules described herein. In particular, the invention provides a polynucleotide having a nucleotide sequence representing the portion of SEQ ID NO:1 which consists of positions 1-534 of SEQ ID NO:1, a polynucleotide having a nucleotide sequence representing the portion of SEQ ID NO:1 which consists of positions 535-798 of SEQ ID NO:1, a polynucleotide having a nucleotide sequence representing the portion of SEQ ID NO:3 which consists of positions 1-685 of SEQ ID NO:3, a polynucleotide having a nucleotide sequence representing the portion of SEQ ID NO:3 which consists of positions 686-1210 of SEQ ID NO:3, a polynucleotide having a nucleotide sequence representing the portion of SEQ ID NO:5 which consists of positions 1-414 of SEQ ID NO:5, and a polynucleotide having a nucleotide sequence representing the portion of SEQ ID NO:5 which consists of positions 415-642 of SEQ ID NO:5.

Further, the invention includes a polynucleotide comprising a sequence at least 95% identical to any portion of at least about 30 contiguous nucleotides, preferably at least about 50 nucleotides, of the sequence from nucleotides 1 to nucleotide 1150 in FIGS. 1A-B (SEQ ID NO:1), from nucleotides 1 to nucleotide 1854 in FIGS. 2A-C (SEQ ID

NO:3), and from nucleotides 1 to nucleotide 726 in FIG. 3 (SEQ ID NO:5).

More generally, by a fragment of an isolated nucleic acid molecule having the nucleotide sequence of the deposited cDNA or the nucleotide sequences shown in FIGS. 1A-B (SEQ ID NO:1), in FIGS. 2A-C (SEQ ID NO:3), or in FIG. 3 (SEQ ID NO:5) is intended fragments at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length which are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments 50-300 nt in length are also useful according to the present invention as are fragments corresponding to most, if not all, of the nucleotide sequence of the deposited cDNA or as shown in FIGS. 1A-B (SEQ ID NO:1), in FIGS. 2A-C (SEQ ID NO:3), and/or FIG. 3 (SEQ ID NO:5). By a fragment at least 20 nt in length, for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequences of the deposited cDNAs or the nucleotide sequences as shown in FIGS. 1A-B (SEQ ID NO:1), in FIGS. 2A-C (SEQ ID NO:3), or in FIG. 3 (SEQ ID NO:5). Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding epitope-bearing portions of the PGRP-K, PGRP-W, and/or PGRP-C polypeptides as identified in FIG. 5 and Table II, FIG. 6 and Table III, and FIG. 7 and Table IV, respectively, and described in more detail below.

In another aspect, the invention provides isolated nucleic acid molecules comprising polynucleotides which hybridizes under stringent hybridization conditions to a portion of the polynucleotide in a nucleic acid molecule of the invention described above, for instance, the cDNA clones contained in ATCC Deposit numbers 203564, 203563, or 209683, respectively, deposited on Dec. 23, 1998, and Mar. 20, 1998. By "stringent hybridization conditions" is intended overnight incubation at 42° C. in a solution comprising: 50% formamide, 5×SSC (750 mM NaCl, 75 mM trisodium citrate), 75 mM sodium phosphate (pH 7.6), 5×Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1×SSC at about 65° C.

By a polynucleotide which hybridizes to a "portion" of a polynucleotide is intended a polynucleotide (either DNA or RNA) hybridizing to at least about 15 nucleotides (nt), and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably about 30-70 (e.g., 50) nt of the reference polynucleotide. These are useful as diagnostic probes and primers as discussed above and in more detail below.

By a portion of a polynucleotide of "at least 20 nt in length," for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence of the reference polynucleotides (e.g., the deposited cDNA or the nucleotide sequence as shown in FIGS. 1A-B (SEQ ID NO:1), in FIGS. 2A-C (SEQ ID NO:3), and/or in FIG. 3 (SEQ ID NO:5)). Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3' terminal poly(A) tract of the PGRP-K, PGRP-W, or PGRP-C cDNA shown in FIGS. 1A-B (SEQ ID NO:1), in FIGS. 2A-C (SEQ ID NO:3), and/or FIG. 3 (SEQ ID NO:5)), or to a complementary stretch of T (or U) residues, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

As indicated, nucleic acid molecules of the present invention which encode a PGRP-K polypeptide may include, but

are not limited to those encoding the amino acid sequence of the PGRP-like domain of the polypeptide, by itself; and the coding sequence for the PGRP-like domain of the polypeptide and additional sequences, such as a pre-, or pro- or prepro-protein sequence.

As indicated, nucleic acid molecules of the present invention which encode a PGRP-W polypeptide may include, but are not limited to those encoding the amino acid sequence of the PGRP-like domain of the polypeptide, by itself; and the coding sequence for the PGRP-like domain of the polypeptide and additional sequences, such as a pre-, or pro- or prepro-protein sequence.

As indicated, nucleic acid molecules of the present invention which encode a PGRP-C polypeptide may include, but are not limited to those encoding the amino acid sequence of the PGRP-like domain of the polypeptide, by itself; and the coding sequence for the PGRP-like domain of the polypeptide and additional sequences, such as a pre-, or pro or prepro-protein sequence.

Also encoded by nucleic acids of the invention are the above protein sequences together with additional, non-coding sequences, including for example, but not limited to introns and non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals, for example—ribosome binding and stability of mRNA; an additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities.

Thus, the sequence encoding the polypeptide may be fused to a marker sequence, such as a sequence encoding a peptide which facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the marker amino acid sequence is a hexahistidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, Calif., 91311), among others, many of which are commercially available. As described in Gentz et al., *Proc. Natl. Acad. Sci. USA* 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. The "HA" tag is another peptide useful for purification which corresponds to an epitope derived from the influenza hemagglutinin protein, which has been described by Wilson et al., *Cell* 37: 767 (1984). As discussed below, other such fusion proteins include either the PGRP-K, PGRP-W, or the PGRP-C fused to Fc at the N- or C-terminus.

#### Variant and Mutant Polynucleotides

The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode portions, analogs or derivatives of either the PGRP-K, PGRP-W, or PGRP-C proteins. Variants may occur naturally, such as a natural allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985). Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

Such variants include those produced by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding regions, non-coding regions, or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of either the PGRP-K, PGRP-W, and/or PGRP-C proteins or portions

thereof. Also especially preferred in this regard are conservative substitutions.

Most highly preferred are nucleic acid molecules encoding the PGRP-like domain 1 of the protein having the amino acid sequence shown in FIGS. 1A-B (SEQ ID NO:2) or the PGRP-like domain of the PGRP-K amino acid sequence encoded by the deposited cDNA clone, the nucleic acid molecules encoding the PGRP-like domain of the protein having the amino acid sequence shown in FIGS. 2A-C (SEQ ID NO:4) or the PGRP-like domain of the PGRP-W amino acid sequence encoded by the deposited cDNA clone, or the nucleic acid molecules encoding the PGRP-like domain of the protein having the amino acid sequence shown in FIG. 3 (SEQ ID NO:6) or the PGRP-like domain of the PGRP-C amino acid sequence encoded by the deposited cDNA clone. Further embodiments include an isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical to a polynucleotide selected from the group consisting of: (a) nucleotide sequences encoding either the PGRP-K, PGRP-W, or PGRP-C polypeptides having the complete amino acid sequences in FIGS. 1A-B (SEQ ID NO:2), in FIGS. 2A-C (SEQ ID NO:4), and/or in FIG. 3 (SEQ ID NO:6); (b) a nucleotide sequence encoding the predicted PGRP-like domain of the PGRP-K, PGRP-W, and PGRP-C polypeptides having the amino acid sequences at positions 72-323 in FIGS. 1A-B (SEQ ID NO:2), the amino acid sequences at positions 156-407 in FIGS. 2A-C (SEQ ID NO:4), and the amino acid sequence at positions 102-353 in FIG. 3 (SEQ ID NO:6), respectively; (c) a nucleotide sequence encoding the PGRP-K, PGRP-W, or PGRP-C polypeptides having the complete amino acid sequences encoded by the cDNA clones contained in ATCC Numbers 203564, 203563, and 209683, respectively, deposited on Dec. 23, 1998, and Mar. 20, 1998; (d) a nucleotide sequence encoding the PGRP-like domain of the PGRP-K, PGRP-W, or PGRP-C polypeptides having the amino acid sequences encoded by the cDNA clones contained in ATCC Numbers 203564, 203563, and 209683, respectively, deposited on Dec. 23, 1998, and Mar. 20, 1998; and (e) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c) or (d) above.

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a PGRP-K, a PGRP-W, or a PGRP-C polypeptide is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence(s) except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding either the PGRP-K, PGRP-W, or PGRP-C polypeptides. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular nucleic acid molecule is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the nucleotide sequences shown in

FIGS. 1A-B, FIGS. 2A-C, and/or FIG. 3 or to the nucleotide sequences of the deposited cDNA clones can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, Wis. 53711). Bestfit uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics* 2:482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

The present application is directed to nucleic acid molecules at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences shown in FIGS. 1A-B (SEQ ID NO:1), FIGS. 2A-C (SEQ ID NO:3), and/or FIG. 3 (SEQ ID NO:5) or to the nucleic acid sequence(s) of the deposited cDNAs, irrespective of whether they encode a polypeptide having PGRP-K, PGRP-W, or PGRP-C activities, respectively. This is because even where a particular nucleic acid molecule does not encode a polypeptide having either PGRP-K, PGRP-W, or PGRP-C activities, one of skill in the art would still know how to use the nucleic acid molecules, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having either PGRP-K, PGRP-W, or PGRP-C activity include, inter alia, (1) isolating the PGRP-K, PGRP-W, or PGRP-C genes or allelic variants thereof in cDNA libraries; (2) in situ hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the PGRP-K, PGRP-W, or PGRP-C genes, as described in Verma et al., *Human Chromosomes: A Manual of Basic Techniques*, Pergamon Press, New York (1988); and Northern Blot analysis for detecting either PGRP-K, PGRP-W, or PGRP-C mRNA expression in specific tissues.

Preferred, however, are nucleic acid molecules having sequences at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences shown in FIGS. 1A-B (SEQ ID NO:1), FIGS. 2A-C (SEQ ID NO:3), and/or FIG. 3 (SEQ ID NO:5), or to the nucleic acid sequence of the deposited cDNAs which do, in fact, encode a polypeptide(s) having either PGRP-K, PGRP-W, or PGRP-C protein activity. By "a polypeptide having PGRP-K, PGRP-W, or PGRP-C activity" is intended polypeptides exhibiting activity similar, but not necessarily identical, to an activity of the PGRP-K, PGRP-W, or PGRP-C proteins of the invention, as measured in a particular biological assay. For example, the PGRP-K, PGRP-W, or PGRP-C proteins of the present invention bind insoluble peptidoglycan. An insoluble peptidoglycan binding assay for measuring the ability of a protein to bind to insoluble peptidoglycan can be performed by using reagents well known and commonly available in the art for detecting this binding ability. For instance, numerous such assays for peptidoglycan binding protein activities are described in the various references in the Background section of this disclosure, above, as well as in example 13. Briefly, such an assay involves collecting insoluble peptidoglycan from an appropriate source (e.g., *Micrococcus luteus*), mixing the insoluble peptidoglycan with a specified amount of the protein in question, and

measuring the concentration of peptidoglycan bound by the protein over a certain period of time. Such insoluble peptidoglycan binding activities as can be measure in this type of assay are useful for identifying proteins that may have a immuno-modulatory effect in the body, and that may act to bind peptidoglycan and/or function in an immune recognition capacity during the infection process of certain bacterial species.

PGRP-K, PGRP-W, and/or PGRP-C proteins bind insoluble peptidoglycan, and are useful for identifying proteins that may have a immuno-modulatory effect in the body, and that may act to bind peptidoglycan and/or function in an immune recognition capacity during the infection process of certain bacterial species. Thus, "a polypeptide having PGRP-K, PGRP-W, and/or PGRP-C protein activity" includes polypeptides that also exhibit any of the same peptidoglycan binding activities in the above-described assays. Although the degree of peptidoglycan binding activity need not be identical to that of the PGRP-K, PGRP-W, and/or PGRP-C proteins, preferably, "a polypeptide having either PGRP-K, PGRP-W, and/or PGRP-C protein activity" will exhibit substantially similar peptidoglycan binding in a given activity as compared to the PGRP-K, PGRP-W, and/or PGRP-C proteins (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity relative to the reference PGRP-K, PGRP-W, and/or PGRP-C proteins). Assays for measuring such activity are known in the art. For example, see Yoshida et al., *JBC*, 271 (23): 13854 (1996); and Kang et al., *PNAS (US)*, 95 (17): 10078 (1998).

Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequences of the deposited cDNAs or the nucleic acid sequences shown in FIGS. 1A-B (SEQ ID NO:1), in FIGS. 2A-C (SEQ ID NO:3), and/or FIG. 3 (SEQ ID NO:5) will encode a polypeptide "having PGRP-K, PGRP-W, and/or PGRP-C protein activity." In fact, since degenerate variants of these nucleotide sequences all encode the same polypeptides, respectively, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode polypeptides having either PGRP-K, PGRP-W, and/or PGRP-C protein activity, respectively. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid), as further described below.

#### Vectors and Host Cells

The present invention also relates to vectors which include the isolated DNA molecules of the present invention, host cells which are genetically engineered with the recombinant vectors, and the production of either PGRP-K, PGRP-W, and/or PGRP-C polypeptides or fragments thereof by recombinant techniques. The vector may be, for example, a phage, plasmid, viral or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells. The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate

precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The DNA insert(s) should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli* lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the PGRP-K, PGRP-W, and/or PGRP-C transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as *Drosophila* S2 and *Spodoptera Sf9* cells; animal cells such as CHO, COS, 293 and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pHE4 (HGS Inc., Provisional Number: PCT/US98/20075); pA2, PO4, and PBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16A, pNH18A, pNH46A, available from Stratagene; and ptc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

Selection of appropriate vectors and promoters for expression in a host cell is a well known procedure and the requisite techniques for expression vector construction, introduction of the vector into the host and expression in the host are routine skills in the art.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., PGRP-K, PGRP-W, and/or PGRP-C coding sequences), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with PGRP-K, PGRP-W, and/or PGRP-C polynucleotides of the invention, respectively, and which activates, alters, and/or amplifies endogenous PGRP-K, PGRP-W, and/or PGRP-C polynucleotides, respectively. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous PGRP-K, PGRP-W, and/or PGRP-C polynucleotide sequences via homologous recombination (see, e.g., U.S. Pat. No. 5,641,670, issued Jun. 24, 1997; International Publication No. WO 96/29411, published Sep. 26, 1996; International Publication No. WO 94/12650, published Aug. 4, 1994; Koller et al., Proc. Natl.

Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

The host cell can be a higher eukaryotic cell, such as a mammalian cell (e.g., a human derived cell), or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. The host strain may be chosen which modulates the expression of the inserted gene sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus expression of the genetically engineered polypeptide may be controlled. Furthermore, different host cells have characteristics and specific mechanisms for the translational and post-translational processing and modification (e.g., glycosylation, phosphorylation, cleavage) of proteins. Appropriate cell lines can be chosen to ensure the desired modifications and processing of the foreign protein expressed.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986).

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals, but also additional heterologous functional regions.

For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art. A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to stabilize and purify proteins. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5 has been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett et al., *J. Molecular Recognition* 8:52-58 (1995) and K. Johanson et al., *J. Biol. Chem.* 270:9459-9471 (1995).

The PGRP-K, PGRP-W, or PGRP-C protein can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lec-

tin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

#### PGRP-K, PGRP-W, and PGRP-C Polypeptides and Fragments

The invention further provides an isolated PGRP-K polypeptide having the amino acid sequence encoded by the deposited cDNA, or the amino acid sequence in FIGS. 1A-B (SEQ ID NO:2), or a peptide or polypeptide comprising a portion of the above polypeptides.

The invention further provides an isolated PGRP-W polypeptide having the amino acid sequence encoded by the deposited cDNA, or the amino acid sequence in FIGS. 2A-C (SEQ ID NO:4), or a peptide or polypeptide comprising a portion of the above polypeptides.

The invention further provides an isolated PGRP-C polypeptide having the amino acid sequence encoded by the deposited cDNA, or the amino acid sequence in FIG. 3 (SEQ ID NO:6), or a peptide or polypeptide comprising a portion of the above polypeptides.

#### Variant and Mutant Polypeptides

To improve or alter the characteristics of either PGRP-K, PGRP-W, and/or PGRP-C polypeptides, protein engineering may be employed. Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or "mutants" including single or multiple amino acid substitutions, deletions, additions or fusion proteins. Such modified polypeptides can show, e.g., enhanced activity or increased stability. In addition, they may be purified in higher yields and show better solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions.

#### N-Terminal and C-Terminal Deletion Mutants

For instance, for many proteins, including the extracellular domain or the mature form(s) of a secreted protein, it is known in the art that one or more amino acids may be deleted from the N-terminus or C-terminus without substantial loss of biological function. For instance, Ron et al., *J. Biol. Chem.*, 268:2984-2988 (1993) reported modified KGF proteins that had heparin binding activity even if 3, 8, or 27 amino-terminal amino acid residues were missing.

In the present case, since the protein of the invention is related to Tag-7, deletions of N-terminal amino acids up to the Gly (G) residue at position 191 in FIG. 1 (SEQ ID NO:2) may retain some biological activity such as cytotoxicity to appropriate target cells. However, even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete PGRP-like domain of the protein generally will be retained when less than the majority of the residues of the complete PGRP domain of the protein are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

In one embodiment, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the PGRP-K polypeptide depicted in FIGS. 1A-B (SEQ ID NO:2) or encoded by the cDNA of the deposited clone. Particularly, in one embodiment, N-terminal deletions of the PGRP-K polypeptide can be described by the general formula m to 243, where m is an integer from 2 to 243 corresponding to the position of the amino acid identified in SEQ ID NO:2 and preferably, corresponds to one of the N-terminal amino acid residues identified in the N-terminal deletions specified herein. In specific embodiments, N-terminal deletions of the PGRP-K polypeptide of the invention comprise, or alternatively, consist of, amino acid residues: G-2 to Q-243; T-3 to Q-243; L-4 to Q-243; P-5 to Q-243; W-6 to Q-243; L-7 to Q-243; L-8 to Q-243; A-9 to Q-243; F-10 to Q-243; F-11 to Q-243; I-12 to Q-243; L-13 to Q-243; G-14 to Q-243; L-15 to Q-243; Q-16 to Q-243; A-17 to Q-243; W-18 to Q-243; D-19 to Q-243; T-20 to Q-243; P-21 to Q-243; T-22 to Q-243; I-23 to Q-243; V-24 to Q-243; S-25 to Q-243; R-26 to Q-243; K-27 to Q-243; E-28 to Q-243; W-29 to Q-243; G-30 to Q-243; A-31 to Q-243; R-32 to Q-243; P-33 to Q-243; L-34 to Q-243; A-35 to Q-243; C-36 to Q-243; R-37 to Q-243; A-38 to Q-243; L-39 to Q-243; L-40 to Q-243; T-41 to Q-243; L-42 to Q-243; P-43 to Q-243; V-44 to Q-243; A-45 to Q-243; Y-46 to Q-243; I-47 to Q-243; I-48 to Q-243; T-49 to Q-243; D-50 to Q-243; Q-51 to Q-243; L-52 to Q-243; P-53 to Q-243; G-54 to Q-243; M-55 to Q-243; Q-56 to Q-243; C-57 to Q-243; Q-58 to Q-243; Q-59 to Q-243; Q-60 to Q-243; S-61 to Q-243; V-62 to Q-243; C-63 to Q-243; S-64 to Q-243; Q-65 to Q-243; M-66 to Q-243; L-67 to Q-243; R-68 to Q-243; G-69 to Q-243; L-70 to Q-243; Q-71 to Q-243; S-72 to Q-243; H-73 to Q-243; S-74 to Q-243; V-75 to Q-243; Y-76 to Q-243; T-77 to Q-243; I-78 to Q-243; G-79 to Q-243; W-80 to Q-243; C-81 to Q-243; D-82 to Q-243; V-83 to Q-243; A-84 to Q-243; Y-85 to Q-243; N-86 to Q-243; F-87 to Q-243; L-88 to Q-243; V-89 to Q-243; G-90 to Q-243; D-91 to Q-243; D-92 to Q-243; G-93 to Q-243; R-94 to Q-243; V-95 to Q-243; Y-96 to Q-243; E-97 to Q-243; G-98 to Q-243; V-99 to Q-243; G-100 to Q-243; W-101 to Q-243; N-102 to Q-243; I-103 to Q-243; Q-104 to Q-243; G-105 to Q-243; L-106 to Q-243; H-107 to Q-243; T-108 to Q-243; Q-109 to Q-243; G-110 to Q-243; Y-111 to Q-243; N-112 to Q-243; N-113 to Q-243; I-114 to Q-243; S-115 to Q-243; L-116 to Q-243; G-117 to Q-243; I-118 to Q-243; A-119 to Q-243; F-120 to Q-243; F-121 to Q-243; G-122 to Q-243; N-123 to Q-243; K-124 to Q-243; I-125 to Q-243; S-126 to Q-243; S-127 to Q-243; S-128 to Q-243; P-129 to Q-243; S-130 to Q-243; P-131 to Q-243; A-132 to Q-243; A-133 to Q-243; L-134 to Q-243; S-135 to Q-243; A-136 to Q-243; A-137 to Q-243; E-138 to Q-243; G-139 to Q-243; L-140 to Q-243; I-141 to Q-243; S-142 to Q-243; Y-143 to Q-243; A-144 to Q-243; I-145 to Q-243; Q-146 to Q-243; K-147 to Q-243; G-148 to Q-243; H-149 to Q-243; L-150 to Q-243; S-151 to Q-243; P-152 to Q-243; R-153 to Q-243; Y-154 to Q-243; I-155 to Q-243; Q-156 to Q-243; P-157 to Q-243; L-158 to Q-243; L-159 to Q-243; L-160 to Q-243; K-161 to Q-243; E-162 to Q-243; E-163 to Q-243; T-164 to Q-243; C-165 to Q-243; L-166 to Q-243; D-167 to Q-243; P-168 to Q-243; Q-169 to Q-243; H-170 to Q-243; P-171 to Q-243; V-172 to Q-243; M-173 to Q-243; P-174 to Q-243; R-175 to Q-243; K-176 to Q-243; V-177 to Q-243; C-178 to Q-243; P-179 to Q-243; N-180 to Q-243; I-181 to Q-243; I-182 to Q-243; K-183 to Q-243; R-184 to Q-243; S-185 to Q-243; A-186 to Q-243; W-187 to Q-243; E-188 to Q-243; A-189 to Q-243; R-190 to Q-243; E-191 to Q-243; T-192 to Q-243; H-193 to Q-243;

C-194 to Q-243; P-195 to Q-243; K-196 to Q-243; M-197 to Q-243; N-198 to Q-243; L-199 to Q-243; P-200 to Q-243; A-201 to Q-243; K-202 to Q-243; Y-203 to Q-243; V-204 to Q-243; I-205 to Q-243; I-206 to Q-243; I-207 to Q-243; H-208 to Q-243; T-209 to Q-243; A-210 to Q-243; G-211 to Q-243; T-212 to Q-243; S-213 to Q-243; C-214 to Q-243; T-215 to Q-243; V-216 to Q-243; S-217 to Q-243; T-218 to Q-243; D-219 to Q-243; C-220 to Q-243; Q-221 to Q-243; T-222 to Q-243; V-223 to Q-243; V-224 to Q-243; R-225 to Q-243; N-226 to Q-243; I-227 to Q-243; Q-228 to Q-243; S-229 to Q-243; F-230 to Q-243; H-231 to Q-243; M-232 to Q-243; D-233 to Q-243; T-234 to Q-243; R-235 to Q-243; N-236 to Q-243; F-237 to Q-243; C-238 to Q-243; of SEQ ID NO:2. Polynucleotides encoding these polypeptides also are provided.

In one embodiment, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the PGRP-W polypeptide depicted in FIGS. 2A-C (SEQ ID NO:4) or encoded by the cDNA of the deposited clone. Particularly, in one embodiment, N-terminal deletions of the PGRP-W polypeptide can be described by the general formula m to 368, where m is an integer from 2 to 368 corresponding to the position of the amino acid identified in SEQ ID NO:4 and preferably, corresponds to one of the N-terminal amino acid residues identified in the N-terminal deletions specified herein. In specific embodiments, N-terminal deletions of the PGRP-W polypeptide of the invention comprise, or alternatively, consist of, amino acid residues: L-2 to H-368; L-3 to H-368; W-4 to H-368; L-5 to H-368; L-6 to H-368; V-7 to H-368; F-8 to H-368; S-9 to H-368; A-10 to H-368; L-11 to H-368; G-12 to H-368; I-13 to H-368; Q-14 to H-368; A-15 to H-368; W-16 to H-368; G-17 to H-368; D-18 to H-368; S-19 to H-368; S-20 to H-368; W-21 to H-368; N-22 to H-368; K-23 to H-368; T-24 to H-368; Q-25 to H-368; A-26 to H-368; K-27 to H-368; Q-28 to H-368; V-29 to H-368; S-30 to H-368; E-31 to H-368; G-32 to H-368; L-33 to H-368; Q-34 to H-368; Y-35 to H-368; L-36 to H-368; F-37 to H-368; E-38 to H-368; N-39 to H-368; I-40 to H-368; S-41 to H-368; Q-42 to H-368; L-43 to H-368; T-44 to H-368; E-45 to H-368; K-46 to H-368; D-47 to H-368; V-48 to H-368; S-49 to H-368; T-50 to H-368; T-51 to H-368; V-52 to H-368; S-53 to H-368; R-54 to H-368; K-55 to H-368; A-56 to H-368; W-57 to H-368; G-58 to H-368; A-59 to H-368; E-60 to H-368; A-61 to H-368; V-62 to H-368; G-63 to H-368; C-64 to H-368; S-65 to H-368; I-66 to H-368; Q-67 to H-368; L-68 to H-368; T-69 to H-368; T-70 to H-368; P-71 to H-368; V-72 to H-368; N-73 to H-368; V-74 to H-368; L-75 to H-368; V-76 to H-368; I-77 to H-368; H-78 to H-368; H-79 to H-368; V-80 to H-368; P-81 to H-368; G-82 to H-368; L-83 to H-368; E-84 to H-368; C-85 to H-368; H-86 to H-368; D-87 to H-368; Q-88 to H-368; T-89 to H-368; V-90 to H-368; C-91 to H-368; S-92 to H-368; Q-93 to H-368; R-94 to H-368; L-95 to H-368; R-96 to H-368; E-97 to H-368; L-98 to H-368; Q-99 to H-368; A-100 to H-368; H-101 to H-368; H-102 to H-368; V-103 to H-368; H-104 to H-368; N-105 to H-368; N-106 to H-368; S-107 to H-368; G-108 to H-368; C-109 to H-368; D-110 to H-368; V-111 to H-368; A-112 to H-368; Y-113 to H-368; N-114 to H-368; F-115 to H-368; L-116 to H-368; V-117 to H-368; G-118 to H-368; D-119 to H-368; D-120 to H-368; G-121 to H-368; R-122 to H-368; V-123 to H-368; Y-124 to H-368; E-125 to H-368; G-126 to H-368; V-127 to H-368; G-128 to H-368; W-129 to H-368; N-130 to H-368; I-131 to H-368; Q-132 to H-368; G-133 to H-368; V-134 to H-368; H-135 to H-368; T-136 to H-368; Q-137 to H-368; G-138 to H-368; Y-139 to H-368; N-140 to H-368;

N-141 to H-368; I-142 to H-368; S-143 to H-368; L-144 to H-368; G-145 to H-368; F-146 to H-368; A-147 to H-368; F-148 to H-368; F-149 to H-368; G-150 to H-368; T-151 to H-368; K-152 to H-368; K-153 to H-368; G-154 to H-368; H-155 to H-368; S-156 to H-368; P-157 to H-368; S-158 to H-368; P-159 to H-368; A-160 to H-368; A-161 to H-368; L-162 to H-368; S-163 to H-368; A-164 to H-368; M-165 to H-368; E-166 to H-368; N-167 to H-368; L-168 to H-368; I-169 to H-368; T-170 to H-368; Y-171 to H-368; A-172 to H-368; V-173 to H-368; Q-174 to H-368; K-175 to H-368; G-176 to H-368; H-177 to H-368; L-178 to H-368; S-179 to H-368; S-180 to H-368; S-181 to H-368; Y-182 to H-368; V-183 to H-368; Q-184 to H-368; P-185 to H-368; L-186 to H-368; L-187 to H-368; G-188 to H-368; K-189 to H-368; G-190 to H-368; E-191 to H-368; N-192 to H-368; C-193 to H-368; L-194 to H-368; A-195 to H-368; P-196 to H-368; R-197 to H-368; Q-198 to H-368; K-199 to H-368; T-200 to H-368; S-201 to H-368; L-202 to H-368; K-203 to H-368; K-204 to H-368; L-205 to H-368; A-206 to H-368; P-207 to H-368; A-208 to H-368; L-209 to H-368; S-210 to H-368; H-211 to H-368; G-212 to H-368; L-213 to H-368; C-214 to H-368; G-215 to H-368; E-216 to H-368; P-217 to H-368; G-218 to H-368; R-219 to H-368; P-220 to H-368; L-221 to H-368; S-222 to H-368; R-223 to H-368; M-224 to H-368; T-225 to H-368; L-226 to H-368; P-227 to H-368; A-228 to H-368; K-229 to H-368; Y-230 to H-368; G-231 to H-368; I-232 to H-368; I-233 to H-368; I-234 to H-368; H-235 to H-368; T-236 to H-368; A-237 to H-368; G-238 to H-368; R-239 to H-368; T-240 to H-368; C-241 to H-368; N-242 to H-368; I-243 to H-368; S-244 to H-368; D-245 to H-368; E-246 to H-368; C-247 to H-368; R-248 to H-368; L-249 to H-368; L-250 to H-368; V-251 to H-368; R-252 to H-368; D-253 to H-368; I-254 to H-368; Q-255 to H-368; S-256 to H-368; F-257 to H-368; Y-258 to H-368; I-259 to H-368; D-260 to H-368; R-261 to H-368; L-262 to H-368; K-263 to H-368; S-264 to H-368; C-265 to H-368; D-266 to H-368; I-267 to H-368; G-268 to H-368; Y-269 to H-368; N-270 to H-368; F-271 to H-368; L-272 to H-368; V-273 to H-368; G-274 to H-368; Q-275 to H-368; D-276 to H-368; G-277 to H-368; A-278 to H-368; I-279 to H-368; Y-280 to H-368; E-281 to H-368; G-282 to H-368; V-283 to H-368; G-284 to H-368; W-285 to H-368; N-286 to H-368; V-287 to H-368; Q-288 to H-368; G-289 to H-368; S-290 to H-368; S-291 to H-368; T-292 to H-368; P-293 to H-368; G-294 to H-368; Y-295 to H-368; D-296 to H-368; D-297 to H-368; I-298 to H-368; A-299 to H-368; L-300 to H-368; G-301 to H-368; I-302 to H-368; T-303 to H-368; F-304 to H-368; M-305 to H-368; G-306 to H-368; T-307 to H-368; F-308 to H-368; T-309 to H-368; G-310 to H-368; I-311 to H-368; P-312 to H-368; P-313 to H-368; N-314 to H-368; A-315 to H-368; A-316 to H-368; A-317 to H-368; L-318 to H-368; E-319 to H-368; A-320 to H-368; A-321 to H-368; Q-322 to H-368; D-323 to H-368; L-324 to H-368; I-325 to H-368; Q-326 to H-368; C-327 to H-368; A-328 to H-368; M-329 to H-368; V-330 to H-368; K-331 to H-368; G-332 to H-368; Y-333 to H-368; L-334 to H-368; T-335 to H-368; P-336 to H-368; N-337 to H-368; Y-338 to H-368; L-339 to H-368; L-340 to H-368; V-341 to H-368; G-342 to H-368; H-343 to H-368; S-344 to H-368; D-345 to H-368; V-346 to H-368; A-347 to H-368; R-348 to H-368; T-349 to H-368; L-350 to H-368; S-351 to H-368; P-352 to H-368; G-353 to H-368; Q-354 to H-368; A-355 to H-368; L-356 to H-368; Y-357 to H-368; N-358 to H-368; I-359 to H-368; I-360 to H-368; S-361 to H-368; T-362 to H-368; W-363 to H-368; of SEQ ID NO:4. Polynucleotides encoding these polypeptides also are provided.

In one embodiment, the present invention further provides polypeptides having one or more residues deleted from the

amino terminus of the amino acid sequence of the PGRP-C polypeptide depicted in FIG. 3 (SEQ ID NO:6) or encoded by the cDNA of the deposited clone. Particularly, in one embodiment, N-terminal deletions of the PGRP-C polypeptide can be described by the general formula m to 196, where m is an integer from 2 to 196 corresponding to the position of the amino acid identified in SEQ ID NO:6 and preferably, corresponds to one of the N-terminal amino acid residues identified in the N-terminal deletions specified herein. In specific embodiments, N-terminal deletions of the PGRP-C polypeptide of the invention comprise, or alternatively, consist of, amino acid residues: S-2 to P-196; R-3 to P-196; R-4 to P-196; S-5 to P-196; M-6 to P-196; L-7 to P-196; L-8 to P-196; A-9 to P-196; W-10 to P-196; A-11 to P-196; L-12 to P-196; P-13 to P-196; S-14 to P-196; L-15 to P-196; L-16 to P-196; R-17 to P-196; L-18 to P-196; G-19 to P-196; A-20 to P-196; A-21 to P-196; Q-22 to P-196; E-23 to P-196; T-24 to P-196; E-25 to P-196; D-26 to P-196; P-27 to P-196; A-28 to P-196; C-29 to P-196; C-30 to P-196; S-31 to P-196; P-32 to P-196; I-33 to P-196; V-34 to P-196; P-35 to P-196; R-36 to P-196; N-37 to P-196; E-38 to P-196; W-39 to P-196; K-40 to P-196; A-41 to P-196; L-42 to P-196; A-43 to P-196; S-44 to P-196; E-45 to P-196; C-46 to P-196; A-47 to P-196; Q-48 to P-196; H-49 to P-196; L-50 to P-196; S-51 to P-196; L-52 to P-196; P-53 to P-196; L-54 to P-196; R-55 to P-196; Y-56 to P-196; V-57 to P-196; V-58 to P-196; V-59 to P-196; S-60 to P-196; H-61 to P-196; T-62 to P-196; A-63 to P-196; G-64 to P-196; S-65 to P-196; S-66 to P-196; C-67 to P-196; N-68 to P-196; T-69 to P-196; P-70 to P-196; A-71 to P-196; S-72 to P-196; C-73 to P-196; Q-74 to P-196; Q-75 to P-196; Q-76 to P-196; A-77 to P-196; R-78 to P-196; N-79 to P-196; V-80 to P-196; Q-81 to P-196; H-82 to P-196; Y-83 to P-196; H-84 to P-196; M-85 to P-196; K-86 to P-196; T-87 to P-196; L-88 to P-196; G-89 to P-196; W-90 to P-196; C-91 to P-196; D-92 to P-196; V-93 to P-196; G-94 to P-196; Y-95 to P-196; N-96 to P-196; F-97 to P-196; L-98 to P-196; I-99 to P-196; G-100 to P-196; E-101 to P-196; D-102 to P-196; G-103 to P-196; L-104 to P-196; V-105 to P-196; Y-106 to P-196; E-107 to P-196; G-108 to P-196; R-109 to P-196; G-110 to P-196; W-111 to P-196; N-112 to P-196; F-113 to P-196; T-114 to P-196; G-115 to P-196; A-116 to P-196; H-117 to P-196; S-118 to P-196; G-119 to P-196; H-120 to P-196; L-121 to P-196; W-122 to P-196; N-123 to P-196; P-124 to P-196; M-125 to P-196; S-126 to P-196; I-127 to P-196; G-128 to P-196; I-129 to P-196; S-130 to P-196; F-131 to P-196; M-132 to P-196; G-133 to P-196; N-134 to P-196; Y-135 to P-196; M-136 to P-196; D-137 to P-196; R-138 to P-196; V-139 to P-196; P-140 to P-196; T-141 to P-196; P-142 to P-196; Q-143 to P-196; A-144 to P-196; I-145 to P-196; R-146 to P-196; A-147 to P-196; A-148 to P-196; Q-149 to P-196; G-150 to P-196; L-151 to P-196; L-152 to P-196; A-153 to P-196; C-154 to P-196; G-155 to P-196; V-156 to P-196; A-157 to P-196; Q-158 to P-196; G-159 to P-196; A-160 to P-196; L-161 to P-196; R-162 to P-196; S-163 to P-196; N-164 to P-196; Y-165 to P-196; L-166 to P-196; L-167 to P-196; K-168 to P-196; G-169 to P-196; H-170 to P-196; R-171 to P-196; D-172 to P-196; V-173 to P-196; Q-174 to P-196; R-175 to P-196; T-176 to P-196; L-177 to P-196; S-178 to P-196; P-179 to P-196; G-180 to P-196; N-181 to P-196; Q-182 to P-196; L-183 to P-196; Y-184 to P-196; H-185 to P-196; L-186 to P-196; I-187 to P-196; Q-188 to P-196; N-189 to P-196; W-190 to P-196; P-191 to P-196; of SEQ ID NO:6. Polynucleotides encoding these polypeptides also are provided.

Further embodiments of the invention are directed to C-terminal deletions of the PGRP-K polypeptide described

by the general formula 1 to n, where n is an integer from 7-242 corresponding to the position of amino acid residues identified in SEQ ID NO:2 and preferably, corresponds to one of the C-terminal amino acid residues identified in the C-terminal deletions specified herein. In specific embodiments, C terminal deletions of the PGRP-K polypeptide of the invention comprise, or alternatively, consist of, amino acid residues: M-1 to Y-242; M-1 to G-241; M-1 to I-240; M-1 to D-239; M-1 to C-238; M-1 to F-237; M-1 to N-236; M-1 to R-235; M-1 to T-234; M-1 to D-233; M-1 to M-232; M-1 to H-231; M-1 to F-230; M-1 to S-229; M-1 to Q-228; M-1 to I-227; M-1 to N-226; M-1 to R-225; M-1 to V-224; M-1 to V-223; M-1 to T-222; M-1 to Q-221; M-1 to C-220; M-1 to D-219; M-1 to T-218; M-1 to S-217; M-1 to V-216; M-1 to T-215; M-1 to C-214; M-1 to S-213; M-1 to T-212; M-1 to G-211; M-1 to A-210; M-1 to T-209; M-1 to H-208; M-1 to I-207; M-1 to I-206; M-1 to I-205; M-1 to V-204; M-1 to Y-203; M-1 to K-202; M-1 to A-201; M-1 to P-200; M-1 to L-199; M-1 to N-198; M-1 to M-197; M-1 to K-196; M-1 to P-195; M-1 to C-194; M-1 to H-193; M-1 to T-192; M-1 to E-191; M-1 to R-190; M-1 to A-189; M-1 to E-188; M-1 to W-187; M-1 to A-186; M-1 to S-185; M-1 to R-184; M-1 to K-183; M-1 to I-182; M-1 to I-181; M-1 to N-180; M-1 to P-179; M-1 to C-178; M-1 to V-177; M-1 to K-176; M-1 to R-175; M-1 to P-174; M-1 to M-173; M-1 to V-172; M-1 to P-171; M-1 to H-170; M-1 to Q-169; M-1 to P-168; M-1 to D-167; M-1 to L-166; M-1 to C-165; M-1 to T-164; M-1 to E-163; M-1 to E-162; M-1 to K-161; M-1 to L-160; M-1 to L-159; M-1 to L-158; M-1 to P-157; M-1 to Q-156; M-1 to I-155; M-1 to Y-154; M-1 to R-153; M-1 to P-152; M-1 to S-151; M-1 to L-150; M-1 to H-149; M-1 to G-148; M-1 to K-147; M-1 to Q-146; M-1 to I-145; M-1 to A-144; M-1 to Y-143; M-1 to S-142; M-1 to I-141; M-1 to L-140; M-1 to G-139; M-1 to E-138; M-1 to A-137; M-1 to A-136; M-1 to S-135; M-1 to L-134; M-1 to A-133; M-1 to A-132; M-1 to P-131; M-1 to S-130; M-1 to P-129; M-1 to S-128; M-1 to S-127; M-1 to S-126; M-1 to I-125; M-1 to K-124; M-1 to N-123; M-1 to G-122; M-1 to F-121; M-1 to F-120; M-1 to A-119; M-1 to I-118; M-1 to G-117; M-1 to L-116; M-1 to S-115; M-1 to I-114; M-1 to N-113; M-1 to N-112; M-1 to Y-111; M-1 to G-110; M-1 to Q-109; M-1 to T-108; M-1 to H-107; M-1 to L-106; M-1 to G-105; M-1 to Q-104; M-1 to I-103; M-1 to N-102; M-1 to W-101; M-1 to G-100; M-1 to V-99; M-1 to G-98; M-1 to E-97; M-1 to Y-96; M-1 to V-95; M-1 to R-94; M-1 to G-93; M-1 to D-92; M-1 to D-91; M-1 to G-90; M-1 to V-89; M-1 to L-88; M-1 to F-87; M-1 to N-86; M-1 to Y-85; M-1 to A-84; M-1 to V-83; M-1 to D-82; M-1 to C-81; M-1 to W-80; M-1 to G-79; M-1 to I-78; M-1 to T-77; M-1 to Y-76; M-1 to V-75; M-1 to S-74; M-1 to H-73; M-1 to S-72; M-1 to Q-71; M-1 to L-70; M-1 to G-69; M-1 to R-68; M-1 to L-67; M-1 to M-66; M-1 to Q-65; M-1 to S-64; M-1 to C-63; M-1 to V-62; M-1 to S-61; M-1 to Q-60; M-1 to Q-59; M-1 to Q-58; M-1 to C-57; M-1 to Q-56; M-1 to M-55; M-1 to G-54; M-1 to P-53; M-1 to L-52; M-1 to Q-51; M-1 to D-50; M-1 to T-49; M-1 to I-48; M-1 to I-47; M-1 to Y-46; M-1 to A-45; M-1 to V-44; M-1 to P-43; M-1 to L-42; M-1 to T-41; M-1 to L-40; M-1 to L-39; M-1 to A-38; M-1 to R-37; M-1 to C-36; M-1 to A-35; M-1 to L-34; M-1 to P-33; M-1 to R-32; M-1 to A-31; M-1 to G-30; M-1 to W-29; M-1 to E-28; M-1 to K-27; M-1 to R-26; M-1 to S-25; M-1 to V-24; M-1 to I-23; M-1 to T-22; M-1 to P-21; M-1 to T-20; M-1 to D-19; M-1 to W-18; M-1 to A-17; M-1 to Q-16; M-1 to L-15; M-1 to G-14; M-1 to L-13; M-1 to I-12; M-1 to F-11; M-1 to F-10; M-1 to A-9; M-1 to L-8; M-1 to L-7; SEQ ID NO:2. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Further embodiments of the invention are directed to C-terminal deletions of the PGRP-W polypeptide described by the general formula 1 to n, where n is an integer from 7-367 corresponding to the position of amino acid residues identified in SEQ ID NO:4 and preferably, corresponds to one of the C-terminal amino acid residues identified in the C-terminal deletions specified herein. In specific embodiments, C terminal deletions of the PGRP-W polypeptide of the invention comprise, or alternatively, consist of, amino acid residues: M-1 to K-367; M-1 to F-366; M-1 to H-365; M-1 to P-364; M-1 to W-363; M-1 to T-362; M-1 to S-361; M-1 to I-360; M-1 to I-359; M-1 to N-358; M-1 to Y-357; M-1 to L-356; M-1 to A-355; M-1 to Q-354; M-1 to G-353; M-1 to P-352; M-1 to S-351; M-1 to L-350; M-1 to T-349; M-1 to R-348; M-1 to A-347; M-1 to V-346; M-1 to D-345; M-1 to S-344; M-1 to H-343; M-1 to G-342; M-1 to V-341; M-1 to L-340; M-1 to L-339; M-1 to Y-338; M-1 to N-337; M-1 to P-336; M-1 to T-335; M-1 to L-334; M-1 to Y-333; M-1 to G-332; M-1 to K-331; M-1 to V-330; M-1 to M-329; M-1 to A-328; M-1 to C-327; M-1 to Q-326; M-1 to I-325; M-1 to L-324; M-1 to D-323; M-1 to Q-322; M-1 to A-321; M-1 to A-320; M-1 to E-321; M-1 to L-318; M-1 to A-317; M-1 to A-316; M-1 to A-315; M-1 to N-314; M-1 to P-313; M-1 to P-312; M-1 to I-311; M-1 to G-310; M-1 to T-309; M-1 to F-308; M-1 to T-307; M-1 to G-306; M-1 to M-305; M-1 to F-304; M-1 to T-303; M-1 to I-302; M-1 to G-301; M-1 to L-300; M-1 to A-299; M-1 to I-298; M-1 to D-297; M-1 to D-296; M-1 to Y-295; M-1 to G-294; M-1 to P-293; M-1 to T-292; M-1 to S-291; M-1 to S-290; M-1 to G-289; M-1 to Q-288; M-1 to V-287; M-1 to N-286; M-1 to W-285; M-1 to G-284; M-1 to V-283; M-1 to G-282; M-1 to E-281; M-1 to Y-280; M-1 to I-279; M-1 to A-278; M-1 to G-277; M-1 to D-276; M-1 to Q-275; M-1 to G-274; M-1 to V-273; M-1 to L-272; M-1 to F-271; M-1 to N-270; M-1 to Y-269; M-1 to G-268; M-1 to I-267; M-1 to D-266; M-1 to C-265; M-1 to S-264; M-1 to K-263; M-1 to L-262; M-1 to R-261; M-1 to D-260; M-1 to I-259; M-1 to Y-258; M-1 to F-257; M-1 to S-256; M-1 to Q-255; M-1 to I-254; M-1 to D-253; M-1 to R-252; M-1 to V-251; M-1 to L-250; M-1 to L-249; M-1 to R-248; M-1 to C-247; M-1 to E-246; M-1 to D-245; M-1 to S-244; M-1 to I-243; M-1 to N-242; M-1 to C-241; M-1 to T-240; M-1 to R-239; M-1 to G-238; M-1 to A-237; M-1 to T-236; M-1 to H-235; M-1 to I-234; M-1 to I-233; M-1 to I-232; M-1 to G-231; M-1 to Y-230; M-1 to K-229; M-1 to A-228; M-1 to P-227; M-1 to L-226; M-1 to T-225; M-1 to M-224; M-1 to R-223; M-1 to S-222; M-1 to L-221; M-1 to P-220; M-1 to R-219; M-1 to G-218; M-1 to P-217; M-1 to E-216; M-1 to G-215; M-1 to C-214; M-1 to L-213; M-1 to G-212; M-1 to H-211; M-1 to S-210; M-1 to L-209; M-1 to A-208; M-1 to P-207; M-1 to A-206; M-1 to L-205; M-1 to K-204; M-1 to K-203; M-1 to L-202; M-1 to S-201; M-1 to T-200; M-1 to K-199; M-1 to Q-198; M-1 to R-197; M-1 to P-196; M-1 to A-195; M-1 to L-194; M-1 to C-193; M-1 to N-192; M-1 to E-191; M-1 to G-190; M-1 to K-189; M-1 to G-188; M-1 to L-187; M-1 to L-186; M-1 to P-185; M-1 to Q-184; M-1 to V-183; M-1 to Y-182; M-1 to S-181; M-1 to S-180; M-1 to S-179; M-1 to L-178; M-1 to H-177; M-1 to G-176; M-1 to K-175; M-1 to Q-174; M-1 to V-173; M-1 to A-172; M-1 to Y-171; M-1 to T-170; M-1 to I-169; M-1 to L-168; M-1 to N-167; M-1 to E-166; M-1 to M-165; M-1 to A-164; M-1 to S-163; M-1 to L-162; M-1 to A-161; M-1 to A-160; M-1 to P-159; M-1 to S-158; M-1 to P-157; M-1 to S-156; M-1 to H-155; M-1 to G-154; M-1 to K-153; M-1 to K-152; M-1 to T-151; M-1 to G-150; M-1 to F-149; M-1 to F-148; M-1 to A-147; M-1 to F-146; M-1 to G-145; M-1 to L-144; M-1 to S-143; M-1 to I-142; M-1 to N-141; M-1 to N-140; M-1 to Y-139; M-1 to

G-138; M-1 to Q-137; M-1 to T-136; M-1 to H-135; M-1 to V-134; M-1 to G-133; M-1 to Q-132; M-1 to I-131; M-1 to N-130; M-1 to W-129; M-1 to G-128; M-1 to V-127; M-1 to G-126; M-1 to E-125; M-1 to Y-124; M-1 to V-123; M-1 to R-122; M-1 to G-121; M-1 to D-120; M-1 to D-119; M-1 to G-118; M-1 to V-117; M-1 to L-116; M-1 to F-115; M-1 to N-114; M-1 to Y-113; M-1 to A-112; M-1 to V-111; M-1 to D-110; M-1 to C-109; M-1 to G-108; M-1 to S-107; M-1 to N-106; M-1 to N-105; M-1 to H-104; M-1 to V-103; M-1 to H-102; M-1 to H-101; M-1 to A-100; M-1 to Q-99; M-1 to L-98; M-1 to E-97; M-1 to R-96; M-1 to L-95; M-1 to R-94; M-1 to Q-93; M-1 to S-92; M-1 to C-91; M-1 to V-90; M-1 to T-89; M-1 to Q-88; M-1 to D-87; M-1 to H-86; M-1 to C-85; M-1 to E-84; M-1 to L-83; M-1 to G-82; M-1 to P-81; M-1 to V-80; M-1 to H-79; M-1 to H-78; M-1 to I-77; M-1 to V-76; M-1 to L-75; M-1 to V-74; M-1 to N-73; M-1 to V-72; M-1 to P-71; M-1 to T-70; M-1 to T-69; M-1 to L-68; M-1 to Q-67; M-1 to I-66; M-1 to S-65; M-1 to C-64; M-1 to G-63; M-1 to V-62; M-1 to A-61; M-1 to E-60; M-1 to A-59; M-1 to G-58; M-1 to W-57; M-1 to A-56; M-1 to K-55; M-1 to R-54; M-1 to S-53; M-1 to V-52; M-1 to T-51; M-1 to T-50; M-1 to S-49; M-1 to V-48; M-1 to D-47; M-1 to K-46; M-1 to E-45; M-1 to T-44; M-1 to L-43; M-1 to Q-42; M-1 to S-41; M-1 to I-40; M-1 to N-39; M-1 to E-38; M-1 to F-37; M-1 to L-36; M-1 to Y-35; M-1 to Q-34; M-1 to L-33; M-1 to G-32; M-1 to E-31; M-1 to S-30; M-1 to V-29; M-1 to Q-28; M-1 to K-27; M-1 to A-26; M-1 to Q-25; M-1 to T-24; M-1 to K-23; M-1 to N-22; M-1 to W-21; M-1 to S-20; M-1 to S-19; M-1 to D-18; M-1 to G-17; M-1 to W-16; M-1 to A-15; M-1 to Q-14; M-1 to I-13; M-1 to G-12; M-1 to L-11; M-1 to A-10; M-1 to S-9; M-1 to F-8; M-1 to V-7; of SEQ ID NO:4. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Further embodiments of the invention are directed to C-terminal deletions of the PGRP-C polypeptide described by the general formula 1 to n, where n is an integer from 7-196 corresponding to the position of amino acid residues identified in SEQ ID NO:6 and preferably, corresponds to one of the C-terminal amino acid residues identified in the C-terminal deletions specified herein. In specific embodiments, C terminal deletions of the PGRP-C polypeptide of the invention comprise, or alternatively, consist of, amino acid residues: M-1 to S-195; M-1 to R-194; M-1 to Y-193; M-1 to H-192; M-1 to P-191; M-1 to W-190; M-1 to N-189; M-1 to Q-188; M-1 to I-187; M-1 to L-186; M-1 to H-185; M-1 to Y-184; M-1 to L-183; M-1 to Q-182; M-1 to N-181; M-1 to G-180; M-1 to P-179; M-1 to S-178; M-1 to L-177; M-1 to T-176; M-1 to R-175; M-1 to Q-174; M-1 to V-173; M-1 to D-172; M-1 to R-171; M-1 to H-170; M-1 to G-169; M-1 to K-168; M-1 to L-167; M-1 to V-166; M-1 to Y-165; M-1 to N-164; M-1 to S-163; M-1 to R-162; M-1 to L-161; M-1 to A-160; M-1 to G-159; M-1 to Q-158; M-1 to A-157; M-1 to V-156; M-1 to G-155; M-1 to C-154; M-1 to A-153; M-1 to L-152; M-1 to L-151; M-1 to G-150; M-1 to Q-149; M-1 to A-148; M-1 to A-147; M-1 to R-146; M-1 to I-145; M-1 to A-144; M-1 to Q-143; M-1 to P-142; M-1 to T-141; M-1 to P-140; M-1 to V-139; M-1 to R-138; M-1 to D-137; M-1 to M-136; M-1 to Y-135; M-1 to N-134; M-1 to G-133; M-1 to M-132; M-1 to F-131; M-1 to S-130; M-1 to I-129; M-1 to G-128; M-1 to I-127; M-1 to S-126; M-1 to M-125; M-1 to P-124; M-1 to N-123; M-1 to W-122; M-1 to L-121; M-1 to H-120; M-1 to G-119; M-1 to S-118; M-1 to H-117; M-1 to A-116; M-1 to G-115; M-1 to T-114; M-1 to F-113; M-1 to N-112; M-1 to W-111; M-1 to G-110; M-1 to R-109; M-1 to G-108; M-1 to E-107; M-1 to Y-106; M-1 to V-105; M-1 to L-104; M-1 to G-103; M-1 to D-102; M-1 to E-101; M-1 to G-100; M-1 to I-99; M-1 to L-98; M-1 to

F-97; M-1 to N-96; M-1 to Y-95; M-1 to G-94; M-1 to V-93; M-1 to D-92; M-1 to C-91; M-1 to W-90; M-1 to G-89; M-1 to L-88; M-1 to T-87; M-1 to K-86; M-1 to M-85; M-1 to H-84; M-1 to Y-83; M-1 to H-82; M-1 to Q-81; M-1 to V-80; M-1 to N-79; M-1 to R-78; M-1 to A-77; M-1 to Q-76; M-1 to Q-75; M-1 to Q-74; M-1 to C-73; M-1 to S-72; M-1 to A-71; M-1 to P-70; M-1 to T-69; M-1 to N-68; M-1 to C-67; M-1 to S-66; M-1 to S-65; M-1 to G-64; M-1 to A-63; M-1 to T-62; M-1 to H-61; M-1 to S-60; M-1 to V-59; M-1 to V-58; M-1 to V-57; M-1 to Y-56; M-1 to R-55; M-1 to L-54; M-1 to P-53; M-1 to L-52; M-1 to S-51; M-1 to L-50; M-1 to H-49; M-1 to Q-48; M-1 to A-47; M-1 to C-46; M-1 to E-45; M-1 to S-44; M-1 to A-43; M-1 to L-42; M-1 to A-41; M-1 to K-40; M-1 to W-39; M-1 to E-38; M-1 to N-37; M-1 to R-36; M-1 to P-35; M-1 to V-34; M-1 to I-33; M-1 to P-32; M-1 to S-31; M-1 to C-30; M-1 to C-29; M-1 to A-28; M-1 to P-27; M-1 to D-26; M-1 to E-25; M-1 to T-24; M-1 to E-23; M-1 to Q-22; M-1 to A-21; M-1 to A-20; M-1 to G-19; M-1 to L-18; M-1 to R-17; M-1 to L-16; M-1 to L-15; M-1 to S-14; M-1 to P-13; M-1 to L-12; M-1 to A-11; M-1 to W-10; M-1 to A-9; M-1 to L-8; M-1 to L-7; of SEQ ID NO:6. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Further embodiments of the invention are directed to polypeptide fragments comprising, or alternatively, consisting of, amino acids described by the general formula m to n, where m and n are integers corresponding to any one of the amino acid residues specified above for these symbols, respectively. Polynucleotides encoding such polypeptides are also provided.

It will be recognized in the art that some amino acid sequences of the PGRP-K, the PGRP-W, and/or the PGRP-C proteins can be varied without significant effect to the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on each protein which determine activity. Thus, the invention further includes variations of the PGRP-K protein, the PGRP-W protein, and/or the PGRP-C protein which show substantial PGRP-like activity or which include regions of either the PGRP-K protein, the PGRP-W protein, and/or the PGRP-C protein such as the polypeptide portions discussed below. Such mutants include deletions, insertions, inversions, repeats, and type substitutions. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science* 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. (Cunningham and Wells, *Science*

244:1081-1085 (1989).) The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues is Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

The resulting constructs can be routinely screened for activities or functions described throughout the specification and known in the art. Preferably, the resulting constructs have an increased and/or a decreased PGRP activity or function, while the remaining activities or functions are maintained. More preferably, the resulting constructs have more than one increased and/or decreased PGRP activity or function, while the remaining activities or functions are maintained.

Besides conservative amino acid substitution, variants of PGRP-K, PGRP-W, or PGRP-C include: (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as, for example, an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, PGRP-K, PGRP-W, or PGRP-C polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. (Pinckard et al., *Clin. Exp. Immunol.* 2:331-340 (1967); Robbins et al., *Diabetes* 36: 838-845 (1987); Cleland et al., *Crit. Rev. Therapeutic Drug Carrier Systems* 10:307-377 (1993).)

The resulting constructs can be routinely screened for activities or functions described throughout the specification and known in the art. Preferably, the resulting constructs have an increased and/or decreased PGRP activity or function, while the remaining activities or functions are maintained. More preferably, the resulting constructs have more than one increased and/or decreased PGRP activity or function, while the remaining activities or functions are maintained.

Additionally, more than one amino acid (e.g., 2, 3, 4, 5, 6, 7, 8, 9 and 10) can be replaced with the substituted amino acids as described above (either conservative or nonconservative). The substituted amino acids can occur in the full length, mature, or proprotein form of the PGRP-K, PGRP-W, or PGRP-C proteins, respectively, as well as the

N- and C-terminal deletion mutants, having the general formula m-n, as discussed above.

A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of a PGRP-K, PGRP-W, or PGRP-C polypeptide having an amino acid sequence which contains at least one amino acid substitution, but not more than 50 amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a polypeptide to have an amino acid sequence which comprises the amino acid sequence of a PGRP-K, PGRP-W, or PGRP-C polypeptide, which contains at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid substitutions. In specific embodiments, the number of additions, substitutions, and/or deletions in the amino acid sequence of FIG. 1 or fragments thereof (e.g., the mature form and/or other fragments described herein), is 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, conservative amino acid substitutions are preferable.

Thus, the fragment, derivative or analog of the polypeptides of FIGS. 1A-B (SEQ ID NO:2), FIGS. 2A-C (SEQ ID NO:4), and/or FIG. 3 (SEQ ID NO:6) or that are encoded by the deposited cDNAs, may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the either the PGRP-K, PGRP-W, and/or PGRP-C polypeptides are fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the full length polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the either the PGRP-K, PGRP-W, and/or PGRP-C polypeptides or proprotein sequences. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Of particular interest are substitutions of charged amino acids with another charged amino acid and with neutral or negatively charged amino acids. The latter results in polypeptides with reduced positive charge to improve the characteristics of either the PGRP-K, PGRP-W, and/or PGRP-C polypeptides. The prevention of aggregation is highly desirable. Aggregation of polypeptides not only results in a loss of activity but can also be problematic when preparing pharmaceutical formulations, because they can be immunogenic. (Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36:838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993)).

The replacement of amino acids can also change the selectivity of binding to cell surface receptors. For example, Ostade et al. (Nature 361:266-268 (1993)) describes certain mutations resulting in selective binding of TNF- $\alpha$  to only one of the two known types of TNF receptors. Thus, the PGRP-K, PGRP-W, and/or PGRP-C proteins of the present invention may include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation.

As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not

significantly affect the folding or activity of the protein (see Table 1).

TABLE 1

Conservative Amino Acid Substitutions	
Aromatic	Phenylalanine Tryptophan Tyrosine
Hydrophobic	Leucine Isoleucine Valine
Polar	Glutamine Asparagine
Basic	Arginine Lysine Histidine
Acidic	Aspartic Acid Glutamic Acid
Small	Alanine Serine Threonine Methionine Glycine

In specific embodiments, the number of substitutions, additions or deletions in the amino acid sequence of FIGS. 1A-B (SEQ ID NO:2), FIGS. 2A-C (SEQ ID NO:4), and/or FIG. 3 (SEQ ID NO:6), and/or any of the polypeptide fragments described herein (e.g., PGRP-like domain) is 100, 90, 80, 75, 70, 60, 50, 40, 35, 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or 150-100, 100-50, 50-20, 20-10, 5-10, 1-5, 1-3 or 1-2.

Amino acids in the PGRP-K, PGRP-W, and/or PGRP-C polypeptides of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, Science 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as receptor binding in vitro. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith et al., J. Mol. Biol. 224:899-904 (1992) and de Vos et al., Science 255:306-312 (1992)).

The polypeptides of the present invention also include the polypeptides encoded by the deposited cDNAs; the polypeptide of FIGS. 1A-B (SEQ ID NO:2), the polypeptides of FIGS. 2A-C (SEQ ID NO:4), and/or the polypeptides of FIG. 3 (SEQ ID NO:6); the polypeptide sequence of any of the PGRP-like domains described herein; the polypeptide sequences of FIGS. 1A-B (SEQ ID NO:2), FIGS. 2A-C, and/or FIG. 3 (SEQ ID NO:6), minus a portion, or all of, one or more of the PGRP-like domains described supra; and polypeptides which are at least 80% identical, more preferably at least 85%, 90% or 95% identical, still more preferably at least 96%, 97%, 98% or 99% identical to the polypeptides described above, and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of either a PGRP-K, PGRP-W, and/or PGRP-C polypeptide is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acids of either the PGRP-K, PGRP-W, and/or PGRP-C

proteins. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to one of the reference amino acid sequences, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequences shown in FIGS. 1A-B (SEQ ID NO:2), FIGS. 2A-C (SEQ ID NO:4), and/or FIG. 3 (SEQ ID NO:6), the amino acid sequence encoded by the deposited cDNA clones, respectively, or fragments thereof, can be determined conventionally using known computer programs such as the MegAlign program, which is included in the suite of computer applications contained within the DNASTAR program. When using MegAlign or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

In a specific embodiment, the identity between a reference (query) sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, is determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245 (1990)). Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter. According to this embodiment, if the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction is made to the results to take into consideration the fact that the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. A determination of whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of this embodiment. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject

sequence. For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C-termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are made for the purposes of this embodiment.

Polynucleotides encoding polypeptides that are 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to either the PGRP-K, PGRP-W, and/or PGRP-C polypeptides described herein are also provided.

Among the especially preferred fragments of the invention are fragments characterized by structural or functional attributes of PGRP-K, PGRP-W, and/or PGRP-C. Such fragments include amino acid residues that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet-forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, surface forming regions, and high antigenic index regions (i.e., containing four or more contiguous amino acids having an antigenic index of greater than or equal to 1.5, as identified using the default parameters of the Jameson-Wolf program) of complete (i.e., full-length) PGRP-K (SEQ ID NO:2), PGRP-W (SEQ ID NO:4), and/or PGRP-C (SEQ ID NO:6). Certain preferred regions are those set out in FIG. 5, FIG. 6, or FIG. 7, and include, but are not limited to, regions of the aforementioned types identified by analysis of the amino acid sequence depicted in FIGS. 1A-1B (SEQ ID NO:2), FIGS. 2A-2C (SEQ ID NO:4), and FIG. 3 (SEQ ID NO:6), such preferred regions include; Garnier-Robson predicted alpha-regions, beta-regions, turn-regions, and coil-regions; Chou-Fasman predicted alpha-regions, beta-regions, turn-regions, and coil-regions; Kyte-Doolittle predicted hydrophilic and hydrophobic regions; Eisenberg alpha and beta amphipathic regions; Emami surface-forming regions; and Jameson-Wolf high antigenic index regions, as predicted using the default parameters of these computer programs. Polynucleotides encoding these polypeptides are also encompassed by the invention.

In additional embodiments, the polynucleotides of the invention encode functional attributes of PGRP-K (SEQ ID NO:2), PGRP-W (SEQ ID NO:4), and/or PGRP-C (SEQ ID NO:6). Preferred embodiments of the invention in this regard include fragments that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic

regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions and high antigenic index regions of PGRP-K (SEQ ID NO:2), PGRP-W (SEQ ID NO:4), and/or PGRP-C (SEQ ID NO:6).

The data representing the structural or functional attributes of PGRP-K (SEQ ID NO:2) set forth in FIG. 5 and/or Table II, as described above, was generated using the various modules and algorithms of the DNA\*STAR set on default parameters. The data representing the structural or functional attributes of PGRP-W (SEQ ID NO:4) set forth in FIG. 6 and/or Table III, as described above, was generated using the various modules and algorithms of the DNA\*STAR set on default parameters. The data representing the structural or functional attributes of PGRP-C (SEQ ID NO:6) set forth in FIG. 7 and/or Table IV, as described above, was generated using the various modules and algorithms of the DNA\*STAR set on default parameters. In a preferred embodiment, the data presented in columns VIII, IX, XIII, and XIV of Tables II, III, and IV can be used to determine regions of PGRP-K, PGRP-W, and PGRP-C, respectively, which exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from the data presented in columns VIII, IX, XIII, and/or IV by choosing values which represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response.

Certain preferred regions in these regards are set out in FIGS. 5, 6, and 7, but may, as shown in Tables II, III, and IV, be represented or identified by using tabular representations of the data presented in FIGS. 5, 6, and 7, respectively. The DNA\*STAR computer algorithm used to generate FIGS. 5, 6, and 7 (set on the original default parameters) was used to present the data in FIGS. 5, 6, and 7 in a tabular format (See Tables II, III, and IV). The tabular format of the data in FIGS. 5, 6, and 7 may be used to easily determine specific boundaries of a preferred region.

The above-mentioned preferred regions set out in FIGS. 5, 6, and 7, and in Tables II, III, and IV include, but are not limited to, regions of the aforementioned types identified by analysis of the amino acid sequence set out in FIGS. 1A-1B, FIGS. 2A-2C, and FIG. 3. As set out in FIGS. 5, 6, and 7, and in Tables II, III, and IV, respectively, such preferred regions include Garnier-Robson alpha-regions, beta-regions, turn-regions, and coil-regions, Chou-Fasman alpha-regions, beta-regions, and coil-regions, Kyte-Doolittle hydrophilic regions and hydrophobic regions, Eisenberg alpha- and beta-amphipathic regions, Karplus-Schulz flexible regions, Emini surface-forming regions and Jameson-Wolf regions of high antigenic index.

TABLE II

Res	Pos.													
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Met	1	.	.	B	.	.	.	.	-0.33	.	.	.	-0.40	0.44
Gly	2	.	.	B	.	.	.	.	-0.23	.	.	.	-0.40	0.53
Thr	3	.	.	B	B	.	.	.	-0.66	.	.	.	-0.60	0.43
Leu	4	.	.	B	B	.	.	.	-1.08	.	.	.	-0.60	0.36
Pro	5	.	.	B	B	.	.	.	-1.28	.	.	.	-0.60	0.30
Trp	6	.	.	B	B	.	.	.	-1.38	.	.	.	-0.60	0.21
Leu	7	.	.	B	B	.	.	.	-1.73	.	.	.	-0.60	0.22
Leu	8	.	.	B	B	.	.	.	-2.31	.	.	.	-0.60	0.12
Ala	9	.	.	B	B	.	.	.	-2.31	.	.	.	-0.60	0.08
Phe	10	.	.	B	B	.	.	.	-2.44	.	.	.	-0.60	0.08
Phe	11	.	.	B	B	.	.	.	-2.97	.	.	.	-0.60	0.10
Ile	12	.	.	B	B	.	.	.	-2.16	.	.	.	-0.60	0.08
Leu	13	.	.	B	B	.	.	.	-1.93	.	.	.	-0.60	0.16
Gly	14	.	.	B	.	.	.	C	-1.63	.	.	.	-0.40	0.19
Leu	15	.	.	B	.	.	.	C	-0.93	.	.	.	-0.40	0.28
Gln	16	.	.	B	.	.	.	C	-0.54	.	.	.	-0.40	0.57
Ala	17	.	.	B	T	.	.	.	0.13	.	.	.	-0.20	0.84
Trp	18	.	.	B	T	.	.	.	0.63	.	.	.	-0.05	1.57
Asp	19	.	.	B	.	.	.	C	0.09	.	.	F	0.20	1.31
Thr	20	.	.	B	B	.	.	.	0.04	.	.	F	-0.45	0.91
Pro	21	.	.	B	B	.	.	.	-0.26	.	.	F	-0.45	0.64
Thr	22	.	.	B	B	.	.	.	0.44	.	.	F	-0.15	0.51
Ile	23	.	.	B	B	.	.	.	0.78	.	.	.	-0.30	0.70
Val	24	.	.	B	B	.	.	.	0.78	.	.	.	0.30	0.90
Ser	25	.	A	B	.	.	.	.	0.80	.	.	F	0.90	1.08
Arg	26	.	A	B	.	.	.	.	0.67	.	.	F	0.60	1.63
Lys	27	.	A	B	.	.	.	.	0.39	.	.	F	0.90	2.17
Glu	28	.	A	.	.	T	.	.	1.39	.	.	F	1.30	1.63
Trp	29	.	A	.	.	T	.	.	2.03	.	.	.	1.15	1.63
Gly	30	.	A	.	.	T	.	.	1.52	.	.	.	1.15	1.26
Ala	31	.	A	.	.	T	.	.	0.82	.	.	F	0.85	0.60
Arg	32	.	A	.	.	.	.	C	0.11	.	.	F	-0.25	0.58
Pro	33	.	A	.	.	.	.	C	0.22	.	.	.	-0.10	0.31
Leu	34	.	A	.	.	T	.	.	-0.08	.	.	.	0.70	0.61
Ala	35	.	A	B	.	.	.	.	-0.54	.	.	.	0.30	0.31
Cys	36	.	A	B	.	.	.	.	-0.77	.	.	.	-0.30	0.17
Arg	37	.	A	B	B	.	.	.	-1.19	.	.	.	-0.60	0.17
Ala	38	.	A	B	B	.	.	.	-1.79	.	.	.	-0.60	0.24
Leu	39	.	A	B	B	.	.	.	-1.19	.	.	.	-0.60	0.37
Leu	40	.	A	B	B	.	.	.	-1.46	.	.	.	-0.60	0.29
Thr	41	.	A	B	B	.	.	.	-1.38	.	.	.	-0.60	0.21

TABLE II-continued

Res	(PGRP-K)													
	Pos.													
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Leu	42	.	A	B	B	.	.	.	-1.73	*	.	.	-0.60	0.26
Pro	43	.	A	B	B	.	.	.	-2.03	.	.	.	-0.60	0.50
Val	44	.	.	B	B	.	.	.	-2.11	.	.	.	-0.60	0.24
Ala	45	.	.	B	B	.	.	.	-1.61	.	.	.	-0.60	0.20
Tyr	46	.	.	B	B	.	.	.	-1.30	.	.	.	-0.60	0.19
Ile	47	.	.	B	B	.	.	.	-0.49	*	.	.	-0.60	0.43
Ile	48	.	.	B	B	.	.	.	-1.09	*	.	.	-0.60	0.74
Thr	49	.	.	B	B	.	.	.	-0.44	*	.	.	-0.60	0.39
Asp	50	.	.	B	B	.	.	.	-0.20	.	.	F	-0.11	0.86
Gln	51	.	.	B	.	.	.	.	-0.56	.	.	F	0.28	1.21
Leu	52	.	.	.	.	T	T	C	0.33	.	.	F	0.57	0.83
Pro	53	.	.	.	.	T	T	.	0.56	.	.	F	1.41	0.86
Gly	54	.	.	.	.	T	T	.	0.87	.	.	.	0.40	0.27
Met	55	.	.	.	.	T	T	.	0.87	.	.	.	0.36	0.56
Gln	56	.	.	B	B	.	.	.	0.87	.	.	.	-0.18	0.63
Cys	57	.	.	B	B	.	.	.	1.38	.	.	F	0.08	1.09
Gln	58	.	.	B	B	.	.	.	0.73	.	.	F	0.04	1.48
Gln	59	.	.	B	B	.	.	.	0.41	.	.	F	-0.15	0.63
Gln	60	.	.	B	B	.	.	.	0.71	.	.	F	-0.15	0.63
Ser	61	.	.	B	B	.	.	.	0.71	.	.	F	-0.15	0.49
Val	62	.	.	B	B	.	.	.	0.78	.	.	F	-0.15	0.49
Cys	63	.	.	B	B	.	.	.	-0.03	*	.	.	-0.60	0.28
Ser	64	.	.	B	B	.	.	.	0.08	*	.	.	-0.60	0.17
Gln	65	.	.	B	B	.	.	.	-0.27	*	.	.	-0.30	0.46
Met	66	.	.	B	B	.	.	.	-0.78	*	.	.	-0.30	0.84
Leu	67	.	.	B	B	.	.	.	0.08	*	.	.	-0.30	0.52
Arg	68	.	.	B	B	.	.	.	0.44	*	.	.	-0.30	0.52
Gly	69	.	.	B	.	.	.	.	0.71	*	.	F	0.05	0.70
Leu	70	.	.	.	.	.	.	C	0.41	*	.	F	0.40	1.16
Gln	71	.	.	B	.	T	C	C	0.16	*	.	F	1.05	0.79
Ser	72	.	.	.	.	T	C	C	0.72	*	.	.	0.00	0.59
His	73	.	.	B	.	T	.	.	0.30	.	.	.	-0.05	1.13
Ser	74	.	.	B	.	T	.	.	-0.24	.	.	.	-0.20	0.94
Val	75	.	.	B	B	.	.	.	0.22	.	.	.	-0.60	0.49
Tyr	76	.	.	B	B	.	.	.	-0.07	.	.	.	-0.60	0.36
Thr	77	.	.	B	B	.	.	.	-0.43	.	.	.	-0.60	0.28
Ile	78	.	.	B	B	.	.	.	-0.40	.	.	.	-0.60	0.20
Gly	79	.	.	B	B	.	.	.	-0.96	.	.	.	-0.60	0.22
Trp	80	.	.	B	B	.	.	.	-0.69	.	.	.	-0.60	0.11
Cys	81	.	.	B	B	.	.	.	-0.69	.	.	.	-0.60	0.16
Asp	82	.	.	B	B	.	.	.	-0.38	.	.	.	-0.60	0.25
Val	83	.	.	B	B	.	.	.	-0.19	*	.	.	-0.60	0.39
Ala	84	.	.	B	B	.	.	.	-0.66	*	.	.	-0.60	0.63
Tyr	85	.	.	B	B	.	.	.	-1.22	*	.	.	-0.60	0.31
Asn	86	.	.	B	B	.	.	.	-0.90	*	.	.	-0.60	0.31
Phe	87	.	.	B	B	.	.	.	-0.90	*	.	.	-0.29	0.30
Leu	88	.	.	B	B	.	.	.	-0.04	.	.	.	0.02	0.32
Val	89	.	.	B	B	.	.	.	0.20	.	.	.	1.23	0.34
Gly	90	.	.	.	.	T	T	.	0.56	*	.	F	2.49	0.38
Asp	91	.	.	.	.	T	T	.	-0.30	*	.	F	3.10	0.91
Asp	92	.	.	.	.	T	T	C	0.16	*	.	F	2.59	0.91
Gly	93	.	.	.	.	T	.	C	0.97	*	.	F	2.43	1.44
Arg	94	.	.	B	.	.	.	.	1.48	*	.	F	1.72	1.49
Val	95	.	.	B	.	.	.	.	0.97	*	.	F	1.26	0.89
Tyr	96	.	.	B	.	.	.	.	0.62	*	.	.	0.50	0.66
Glu	97	.	.	B	.	.	.	.	0.33	*	.	.	0.50	0.34
Gly	98	.	.	.	.	T	.	.	0.68	*	.	.	0.00	0.48
Val	99	.	.	.	.	T	.	.	-0.32	*	.	.	0.00	0.49
Gly	100	.	.	.	.	T	.	.	0.53	*	.	.	0.00	0.20
Trp	101	.	.	.	.	.	.	C	0.43	*	.	.	-0.20	0.35
Asn	102	.	.	B	.	.	.	.	-0.38	*	.	.	-0.40	0.46
Ile	103	.	.	B	.	.	.	.	-0.07	*	.	.	-0.40	0.38
Gln	104	.	.	B	.	.	.	.	0.48	*	.	.	-0.40	0.50
Gly	105	.	.	B	.	.	.	.	0.82	*	.	F	-0.25	0.45
Leu	106	.	.	B	.	.	.	.	0.77	*	.	F	-0.10	1.10
His	107	.	.	B	.	.	.	.	0.52	*	.	F	0.05	0.63
Thr	108	.	.	.	.	T	C	.	1.41	*	.	F	0.15	1.00
Gln	109	.	.	.	.	T	T	.	1.41	*	.	F	0.50	1.94
Gly	110	.	.	.	.	T	T	.	0.87	*	.	F	0.80	2.30
Tyr	111	.	.	.	.	T	T	.	1.38	*	.	F	0.50	1.12
Asn	112	.	.	.	.	.	.	C	0.60	*	.	F	-0.05	0.86
Asn	113	.	.	B	B	.	.	.	0.57	*	.	.	-0.60	0.72
Ile	114	.	.	B	B	.	.	.	-0.32	*	.	.	-0.60	0.45

TABLE II-continued

(PGRP-K)														
Pos.														
Res	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Ser	115	.	.	B	B	.	.	.	-0.57	.	.	.	-0.60	0.20
Leu	116	.	.	B	B	.	.	.	-1.02	.	.	.	-0.60	0.12
Gly	117	.	.	B	B	.	.	.	-1.72	.	.	.	-0.60	0.15
Ile	118	.	.	B	B	.	.	.	-2.07	.	.	.	0.60	0.10
Ala	119	.	.	B	B	.	.	.	-1.18	.	.	.	0.60	0.12
Phe	120	.	.	B	B	.	.	.	-0.83	.	.	.	-0.60	0.19
Phe	121	.	.	B	.	.	T	.	-0.91	.	.	.	-0.20	0.55
Gly	122	.	.	B	.	.	T	.	-0.87	.	.	F	0.19	0.38
Asn	123	.	.	.	.	T	T	.	-0.28	.	.	F	0.83	0.59
Lys	124	.	.	.	.	T	T	.	0.01	.	.	F	1.37	0.92
Ile	125	.	.	.	.	T	.	.	0.50	.	.	F	2.16	1.24
Ser	126	.	.	.	.	T	.	.	0.90	.	.	F	2.40	1.20
Ser	127	.	.	.	.	.	.	C	1.03	.	.	F	1.81	0.80
Ser	128	.	.	.	.	.	T	C	0.44	.	.	F	1.32	1.77
Pro	129	.	.	.	.	.	T	C	-0.19	.	.	F	1.68	1.33
Ser	130	.	.	.	.	.	T	C	-0.11	.	.	F	0.84	1.00
Pro	131	.	.	.	.	.	T	C	-0.11	.	.	F	0.45	0.62
Ala	132	.	A	B	.	.	.	.	-0.40	.	.	.	-0.30	0.54
Ala	133	.	A	B	.	.	.	.	-0.69	.	.	.	-0.30	0.40
Leu	134	.	A	B	.	.	.	.	-0.48	.	.	.	-0.60	0.26
Ser	135	.	A	B	.	.	.	.	-0.52	.	.	.	-0.30	0.45
Ala	136	.	A	B	.	.	.	.	-1.12	.	.	.	-0.30	0.44
Ala	137	.	A	B	.	.	.	.	-1.42	.	.	.	-0.30	0.44
Glu	138	A	A	B	.	.	.	.	-1.13	.	.	.	-0.30	0.23
Gly	139	.	.	B	.	.	.	.	-0.57	.	.	.	-0.10	0.31
Leu	140	.	.	B	.	.	.	.	-0.86	.	.	.	-0.40	0.48
Ile	141	.	.	B	.	.	.	.	-1.16	.	.	.	-0.40	0.28
Ser	142	.	.	B	.	.	.	.	-0.57	.	.	.	-0.40	0.20
Tyr	143	.	.	B	.	.	.	.	-0.52	.	.	.	-0.40	0.41
Ala	144	.	.	B	.	.	.	.	-0.52	.	.	.	-0.25	1.18
Ile	145	.	.	B	.	.	.	.	0.26	.	.	.	-0.10	0.87
Gln	146	.	.	B	.	.	T	.	0.33	.	.	.	0.36	0.76
Lys	147	.	.	.	.	T	T	.	0.33	.	.	F	1.17	0.62
Gly	148	.	.	.	.	T	T	.	0.37	.	.	F	1.58	1.18
His	149	.	.	.	.	T	T	.	1.07	.	.	F	2.44	1.05
Leu	150	.	.	.	.	.	.	C	1.71	.	.	F	2.60	1.03
Ser	151	.	.	.	.	.	T	C	0.82	.	.	F	1.64	1.64
Pro	152	.	.	.	.	T	T	.	0.78	.	.	F	1.13	0.84
Arg	153	.	.	.	.	T	T	.	0.91	.	.	.	1.17	1.77
Tyr	154	.	.	B	.	.	T	.	0.13	.	.	.	0.51	2.04
Ile	155	.	.	B	.	.	.	.	0.13	.	.	.	0.05	1.09
Gln	156	.	A	B	.	.	.	.	-0.38	.	.	.	-0.60	0.46
Pro	157	.	A	B	.	.	.	.	-0.12	.	.	.	-0.60	0.24
Leu	158	.	A	B	.	.	.	.	-0.23	.	.	.	-0.60	0.69
Leu	159	.	A	B	.	.	.	.	0.01	.	.	.	0.30	0.69
Leu	160	.	A	B	.	.	.	.	0.59	.	.	F	0.75	0.77
Lys	161	.	A	B	.	.	.	.	-0.08	.	.	F	0.60	1.35
Glu	162	.	A	B	.	.	.	.	-0.68	.	.	F	0.75	0.88
Glu	163	.	A	B	.	.	.	.	0.13	.	.	F	0.75	0.88
Thr	164	.	A	.	.	T	.	.	0.73	.	.	F	1.15	0.73
Cys	165	.	A	.	.	T	.	.	1.54	.	.	F	1.15	0.65
Leu	166	.	A	.	.	T	.	.	1.47	.	.	F	0.85	0.65
Asp	167	.	.	.	.	.	T	C	1.26	.	.	F	0.45	0.62
Pro	168	.	.	.	.	T	T	.	0.40	.	.	F	0.80	1.78
Gln	169	.	.	.	.	T	T	.	0.11	.	.	F	0.80	1.60
His	170	.	.	.	.	.	T	C	0.57	.	.	.	0.30	0.95
Pro	171	.	.	B	.	.	.	.	1.49	.	.	.	-0.40	0.95
Val	172	.	.	B	.	.	.	.	1.53	.	.	.	0.05	1.07
Met	173	.	.	B	.	.	.	.	0.89	.	.	.	0.65	1.58
Pro	174	.	.	B	.	.	.	.	0.22	.	.	.	0.50	0.76
Arg	175	.	.	.	T	.	.	.	0.04	.	.	F	1.05	0.55
Lys	176	.	.	B	.	.	.	.	0.26	.	.	F	0.65	0.85
Val	177	.	.	B	.	.	.	.	0.22	.	.	.	0.97	0.89
Cys	178	.	.	B	.	.	T	.	-0.07	.	.	.	1.04	0.32
Pro	179	.	.	B	.	.	T	.	0.19	.	.	.	0.31	0.11
Asn	180	.	.	B	.	.	T	.	0.19	.	.	.	0.48	0.30
Ile	181	.	.	B	.	.	T	.	-0.16	.	.	.	1.70	1.10
Ile	182	.	.	B	.	.	.	.	0.11	.	.	.	1.18	0.95
Lys	183	.	A	B	.	.	.	.	0.49	.	.	.	0.81	0.60
Arg	184	.	A	B	.	.	.	.	0.70	.	.	F	0.19	0.90
Ser	185	.	A	.	.	.	.	C	0.11	.	.	.	1.12	2.21
Ala	186	.	A	.	.	.	.	C	1.11	.	.	.	0.95	1.12
Trp	187	.	A	.	.	.	.	C	2.00	.	.	.	0.95	1.12

TABLE II-continued

(PGRP-K)														
Pos.														
Res	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Glu	188	.	A	.	.	.	.	C	1.64	.	.	.	0.95	1.45
Ala	189	A	A	.	.	.	.	.	1.50	.	.	F	0.90	2.06
Arg	190	.	A	.	.	T	.	.	1.13	.	.	F	1.30	2.67
Glu	191	.	A	.	.	T	.	.	1.51	.	.	F	1.15	0.83
Thr	192	.	A	.	.	T	.	.	1.84	.	.	F	1.30	1.27
His	193	.	A	.	.	T	.	.	1.24	.	.	F	1.30	1.29
Cys	194	.	.	.	.	.	T	C	1.83	.	.	.	0.90	0.74
Pro	195	.	.	.	.	T	T	.	0.91	.	.	.	0.50	0.82
Lys	196	.	.	.	.	T	T	.	0.70	.	.	.	0.50	0.50
Met	197	.	.	.	.	T	T	.	0.42	.	.	.	0.65	1.44
Asn	198	.	.	.	.	.	.	C	0.50	.	.	.	0.10	0.94
Leu	199	.	.	B	.	.	.	.	0.92	.	.	.	0.50	0.94
Pro	200	.	.	B	.	.	.	.	0.28	.	.	.	0.05	1.49
Ala	201	.	.	.	B	T	.	.	-0.66	.	.	.	-0.20	0.69
Lys	202	.	.	B	B	.	.	.	-0.94	.	.	.	-0.60	0.58
Tyr	203	.	.	B	B	.	.	.	-1.83	.	.	.	-0.60	0.26
Val	204	.	.	B	B	.	.	.	-1.06	.	.	.	-0.60	0.18
Ile	205	.	.	B	B	.	.	.	-1.16	.	.	.	-0.60	0.12
Ile	206	.	.	B	B	.	.	.	-1.16	.	.	.	-0.60	0.12
Ile	207	.	.	B	B	.	.	.	-1.54	.	.	.	-0.60	0.16
His	208	.	.	B	B	.	.	.	-1.61	.	.	.	-0.60	0.22
Thr	209	.	.	B	B	.	.	.	-1.06	.	.	.	-0.60	0.46
Ala	210	.	.	.	B	T	.	.	-0.83	.	.	F	-0.05	0.87
Gly	211	.	.	.	.	T	T	.	-0.26	.	.	F	0.65	0.34
Thr	212	.	.	.	.	T	T	.	-0.22	.	.	F	0.65	0.34
Ser	213	.	.	.	.	T	T	.	-0.49	.	.	F	0.35	0.25
Cys	214	.	.	B	.	.	T	.	-0.49	.	.	.	0.10	0.34
Thr	215	.	.	B	.	.	.	.	0.10	.	.	.	-0.40	0.34
Val	216	.	.	B	.	.	.	.	-0.22	.	.	F	0.82	0.43
Ser	217	.	.	B	.	.	T	.	0.09	.	.	F	0.59	0.43
Thr	218	.	.	B	.	.	T	.	0.08	.	.	F	1.36	0.51
Asp	219	.	.	.	.	T	T	.	-0.11	.	.	F	1.93	0.99
Cys	220	.	.	B	.	.	T	.	-0.66	.	.	F	1.70	0.55
Gln	221	.	.	B	B	.	.	.	0.31	.	.	F	0.53	0.28
Thr	222	.	.	B	B	.	.	.	0.61	.	.	.	0.81	0.33
Val	223	.	.	B	B	.	.	.	0.03	.	.	.	0.04	0.99
Val	224	.	.	B	B	.	.	.	0.03	.	.	.	-0.13	0.40
Arg	225	.	.	B	B	.	.	.	0.40	.	.	.	-0.30	0.48
Asn	226	.	.	B	B	.	.	.	-0.30	.	.	.	-0.30	0.87
Ile	227	.	.	B	B	.	.	.	-0.02	.	.	F	0.00	1.02
Gln	228	.	.	B	B	.	.	.	0.23	.	.	F	-0.15	0.71
Ser	229	.	.	B	B	.	.	.	1.09	.	.	.	-0.60	0.43
Phe	230	.	.	B	B	.	.	.	0.67	.	.	.	0.13	1.04
His	231	.	.	B	B	.	.	.	0.78	.	.	.	0.26	0.86
Met	232	.	.	B	B	.	.	.	1.67	.	.	.	1.29	1.26
Asp	233	.	.	.	.	T	T	.	0.97	.	.	.	2.37	2.34
Thr	234	.	.	.	.	T	T	.	0.60	.	.	F	2.80	1.49
Arg	235	.	.	.	.	T	T	.	1.30	.	.	F	2.37	0.81
Asn	236	.	.	.	.	T	T	.	0.44	.	.	.	2.24	0.81
Phe	237	.	.	.	.	T	.	.	0.70	.	.	.	1.46	0.39
Cys	238	.	.	.	.	T	.	.	0.46	.	.	.	1.18	0.20
Asp	239	.	.	.	.	T	T	.	0.77	.	.	.	0.20	0.19
Ile	240	.	.	.	.	T	T	.	0.27	.	.	.	0.20	0.39
Gly	241	.	.	.	.	T	T	.	-0.12	.	.	.	0.50	0.92
Tyr	242	.	.	B	.	.	T	.	0.19	.	.	.	0.10	0.71
Gln	243	.	.	B	.	.	.	.	0.47	.	.	.	-0.25	1.29

TABLE III

(PGRP-W)														
Pos.														
Res	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Met	1	.	.	.	B	.	.	.	-1.38	.	.	.	-0.60	0.27
Leu	2	A	.	.	B	.	.	.	-1.80	.	.	.	-0.60	0.17
Leu	3	A	.	.	B	.	.	.	-2.27	.	.	.	-0.60	0.11
Trp	4	A	.	.	B	.	.	.	-2.58	.	.	.	-0.60	0.08
Leu	5	A	.	.	B	.	.	.	-2.49	.	.	.	-0.60	0.09

TABLE III-continued

(PGRP-W)														
Pos.														
Res	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Leu	6	A	.	.	B	.	.	.	-2.48	.	.	.	-0.60	0.14
Val	7	A	.	.	B	.	.	.	-2.48	.	.	.	-0.60	0.14
Phe	8	A	.	.	B	.	.	.	-2.01	.	.	.	-0.60	0.14
Ser	9	A	.	.	B	.	.	.	-2.61	.	.	.	-0.60	0.16
Ala	10	A	.	B	B	.	.	.	-1.80	.	.	.	-0.60	0.15
Leu	11	.	.	B	B	.	.	.	-1.58	.	.	.	-0.60	0.31
Gly	12	.	.	B	B	.	.	.	-1.01	.	.	.	-0.60	0.23
Ile	13	.	.	B	B	.	.	.	-0.66	.	.	.	-0.60	0.24
Gln	14	.	.	B	B	.	.	.	-0.36	.	.	.	-0.60	0.29
Ala	15	.	.	B	B	.	.	.	-0.07	.	.	.	-0.30	0.49
Trp	16	.	.	B	B	.	.	.	0.44	.	.	.	-0.30	0.93
Gly	17	.	.	B	.	.	.	C	0.50	.	.	F	0.33	0.72
Asp	18	.	.	.	.	T	T	.	1.39	.	.	F	0.91	0.75
Ser	19	.	.	.	.	.	T	C	1.43	.	.	F	1.14	1.15
Ser	20	.	.	.	.	T	T	.	1.71	.	.	F	2.52	2.32
Trp	21	.	.	.	.	T	T	.	2.00	.	.	F	2.80	2.01
Asn	22	.	.	.	.	.	T	C	1.76	.	.	F	2.32	2.60
Lys	23	.	.	.	.	.	T	C	1.80	.	.	F	1.44	1.96
Thr	24	A	.	.	.	.	T	.	2.10	.	.	F	1.56	3.72
Gln	25	A	.	.	.	.	T	.	1.54	.	.	F	1.84	4.01
Ala	26	.	.	B	.	.	.	.	1.53	.	.	F	1.62	1.49
Lys	27	.	.	B	.	.	.	.	1.53	.	.	F	1.58	1.38
Gln	28	.	.	B	.	.	.	.	1.14	.	.	F	2.14	1.38
Val	29	.	.	B	.	.	T	.	0.64	.	.	F	2.60	1.35
Ser	30	.	.	B	.	.	T	.	0.64	.	.	F	1.89	0.56
Glu	31	.	.	B	.	.	T	.	0.99	.	.	F	1.63	0.56
Gly	32	.	.	B	.	.	T	.	0.13	.	.	F	0.92	1.18
Leu	33	.	A	B	.	.	.	.	-0.57	.	.	.	-0.04	0.73
Gln	34	A	A	.	.	.	.	.	0.29	.	.	.	-0.60	0.36
Tyr	35	.	A	B	.	.	.	.	0.59	.	.	.	-0.60	0.63
Leu	36	.	A	B	.	.	.	.	-0.30	.	.	.	-0.45	1.24
Phe	37	.	A	B	.	.	.	.	-0.26	.	.	.	-0.60	0.50
Glu	38	A	A	.	.	.	.	.	0.56	.	.	.	-0.60	0.43
Asn	39	A	A	.	.	.	.	.	-0.26	.	.	F	-0.15	0.90
Ile	40	A	A	.	.	.	.	.	-0.32	.	.	F	-0.15	0.86
Ser	41	A	A	.	.	.	.	.	0.49	.	.	F	-0.15	0.71
Gln	42	A	A	.	.	.	.	.	1.23	.	.	F	-0.15	0.77
Leu	43	A	A	.	.	.	.	.	1.23	.	.	F	0.60	2.19
Thr	44	A	A	.	.	.	.	.	0.38	.	.	F	0.90	2.73
Glu	45	A	A	.	.	.	.	.	0.97	.	.	F	0.90	1.17
Lys	46	A	A	.	.	.	.	.	0.96	.	.	F	0.90	1.90
Asp	47	.	.	B	B	.	.	.	0.64	.	.	F	0.90	1.90
Val	48	A	.	.	B	.	.	.	0.60	.	.	F	0.90	1.59
Ser	49	.	.	B	B	.	.	.	0.61	.	.	F	0.45	0.59
Thr	50	.	.	B	B	.	.	.	0.72	.	.	F	-0.15	0.47
Thr	51	.	.	B	B	.	.	.	0.72	.	.	F	0.00	1.25
Val	52	.	.	B	B	.	.	.	0.13	.	.	F	0.90	1.86
Ser	53	.	A	B	.	.	.	.	0.70	.	.	F	0.90	1.30
Arg	54	.	A	B	.	.	.	.	0.66	.	.	F	0.45	0.95
Lys	55	.	A	.	.	.	.	C	0.38	.	.	F	0.80	1.26
Ala	56	.	A	.	.	T	.	C	0.69	.	.	.	0.70	0.95
Trp	57	A	A	.	.	.	.	.	0.96	.	.	.	0.60	0.84
Gly	58	A	A	.	.	.	.	.	0.40	.	.	.	0.30	0.43
Ala	59	A	A	.	.	.	.	.	-0.06	.	.	.	-0.60	0.31
Glu	60	A	A	.	.	.	.	.	-0.77	.	.	.	-0.60	0.29
Ala	61	A	.	.	B	.	.	.	-0.48	.	.	.	-0.30	0.16
Val	62	A	.	.	B	.	.	.	-1.08	.	.	.	-0.30	0.21
Gly	63	.	.	.	B	T	.	.	-0.73	.	.	.	0.10	0.09
Cys	64	.	.	B	B	.	.	.	-0.96	.	.	.	-0.60	0.15
Ser	65	.	.	B	B	.	.	.	-1.27	.	.	.	-0.60	0.16
Ile	66	.	.	B	B	.	.	.	-0.99	.	.	.	-0.60	0.24
Gln	67	.	.	B	B	.	.	.	-0.34	.	.	.	-0.60	0.64
Leu	68	.	.	B	B	.	.	.	-0.86	.	.	.	-0.60	0.74
Thr	69	.	.	B	B	.	.	.	-0.19	.	.	F	-0.45	0.78
Thr	70	.	.	B	B	.	.	.	-0.74	.	.	F	-0.45	0.73
Pro	71	.	.	B	B	.	.	.	-0.67	.	.	F	-0.45	0.65
Val	72	.	.	B	B	.	.	.	-1.52	.	.	.	-0.60	0.37
Asn	73	.	.	B	B	.	.	.	-1.60	.	.	.	-0.60	0.19
Val	74	.	.	B	B	.	.	.	-1.32	.	.	.	-0.60	0.09
Leu	75	.	.	B	B	.	.	.	-1.04	.	.	.	-0.60	0.16
Val	76	.	.	B	B	.	.	.	-1.69	.	.	.	-0.60	0.14
Ile	77	.	.	B	B	.	.	.	-1.04	.	.	.	-0.60	0.14
His	78	.	.	B	B	.	.	.	-1.39	.	.	.	-0.60	0.25

TABLE III-continued

(PGRP-W)														
Pos.														
Res	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
His	79	.	.	B	B	.	.	.	-1.34	.	.	.	-0.60	0.34
Val	80	.	.	.	B	.	.	C	-0.53	.	.	.	-0.40	0.40
Pro	81	.	.	.	B	.	.	C	-0.34	.	.	.	-0.10	0.51
Gly	82	.	.	.	.	T	.	.	0.51	.	.	.	0.30	0.20
Leu	63	.	.	.	.	T	.	.	0.54	.	.	.	0.30	0.37
Glu	84	A	.	.	.	.	.	.	0.58	.	.	.	0.50	0.39
Cys	85	A	.	.	.	.	T	.	1.12	.	.	.	0.70	0.69
His	86	A	.	.	.	.	T	.	0.48	.	.	.	0.85	1.21
Asp	87	.	.	.	.	T	T	.	0.16	.	.	F	1.25	0.52
Gln	88	A	.	.	.	.	T	.	0.67	.	.	F	0.25	0.52
Thr	89	.	.	B	B	.	.	.	0.67	.	.	F	0.45	0.51
Val	90	.	.	B	B	.	.	.	1.44	.	.	F	0.45	0.53
Cys	91	.	.	B	B	.	.	.	0.67	.	.	F	0.45	0.60
Ser	92	.	.	B	B	.	.	.	0.78	.	.	F	-0.15	0.34
Gln	93	.	.	B	B	.	.	.	0.78	.	.	F	0.45	0.90
Arg	94	A	.	.	B	.	.	.	0.28	.	.	F	0.90	2.91
Leu	95	A	.	.	B	.	.	.	1.13	.	.	F	0.90	1.79
Arg	96	A	.	.	B	.	.	.	1.21	.	.	F	0.90	1.79
Glu	97	A	A	.	.	.	.	.	1.48	.	.	.	0.60	0.93
Leu	98	A	A	.	.	.	.	.	1.44	.	.	.	0.45	1.53
Gln	99	A	A	.	.	.	.	.	0.48	.	.	.	0.45	1.06
Ala	100	A	A	.	.	.	.	.	1.26	.	.	.	-0.30	0.45
His	101	A	A	.	.	.	.	.	1.14	.	.	.	-0.60	0.75
His	102	A	A	.	.	.	.	.	1.14	.	.	.	-0.60	0.70
Val	103	.	A	B	.	.	.	.	1.66	.	.	.	-0.45	1.11
His	104	.	A	.	.	T	.	.	1.31	.	.	.	0.50	1.09
Asn	105	.	A	.	.	T	.	.	1.23	.	.	F	0.75	0.79
Asn	106	.	.	.	.	T	T	.	1.27	.	.	F	1.40	0.57
Ser	107	.	.	.	.	T	T	.	0.44	.	.	F	2.25	0.70
Gly	108	.	.	.	.	T	T	.	0.71	.	.	F	2.50	0.32
Cys	109	.	.	.	.	T	T	.	0.50	.	.	.	2.10	0.20
Asp	110	.	.	B	B	.	.	.	0.50	.	.	.	0.45	0.24
Val	111	.	.	B	B	.	.	.	-0.20	.	.	.	0.20	0.39
Ala	112	.	.	B	B	.	.	.	-0.71	.	.	.	-0.35	0.63
Tyr	113	.	.	B	B	.	.	.	-1.22	.	.	.	-0.60	0.31
Asn	114	.	.	B	B	.	.	.	-0.90	.	.	.	-0.60	0.31
Phe	115	.	.	B	B	.	.	.	-0.90	.	.	.	-0.29	0.30
Leu	116	.	.	B	B	.	.	.	-0.04	.	.	.	0.02	0.32
Val	117	.	.	B	B	.	.	.	0.20	.	.	.	1.23	0.34
Gly	118	.	.	.	.	T	T	.	0.56	.	.	F	2.49	0.38
Asp	119	.	.	.	.	T	T	.	-0.30	.	.	F	3.10	0.91
Asp	120	.	.	.	.	T	.	C	0.16	.	.	F	2.59	0.91
Gly	121	.	.	.	.	T	.	C	0.97	.	.	F	2.43	1.44
Arg	122	.	.	B	B	.	.	.	1.48	.	.	F	1.52	1.49
Val	123	.	.	B	B	.	.	.	0.97	.	.	F	1.06	0.89
Tyr	124	.	.	B	B	.	.	.	0.62	.	.	.	0.30	0.66
Glu	125	.	.	B	B	.	.	.	0.33	.	.	.	0.30	0.34
Gly	126	.	.	B	B	T	.	.	0.68	.	.	.	-0.20	0.48
Val	127	.	.	B	T	.	.	.	-0.32	.	.	.	-0.20	0.49
Gly	128	.	.	B	T	.	.	.	0.53	.	.	.	-0.20	0.20
Trp	129	.	.	B	.	.	.	C	0.43	.	.	.	-0.40	0.35
Asn	130	.	.	B	B	.	.	.	-0.42	.	.	.	-0.60	0.46
Ile	131	.	.	B	B	.	.	.	-0.11	.	.	.	-0.60	0.35
Gln	132	.	.	B	B	.	.	.	0.43	.	.	.	-0.60	0.45
Gly	133	.	.	B	B	.	.	.	0.78	.	.	.	-0.60	0.40
Val	134	.	.	B	B	.	.	.	0.72	.	.	F	-0.45	0.99
His	135	.	.	B	B	.	.	.	0.48	.	.	F	-0.15	0.57
Thr	136	.	.	B	.	.	T	.	1.37	.	.	F	-0.05	0.90
Gln	137	.	.	B	.	.	T	.	1.37	.	.	F	0.10	1.94
Gly	138	.	.	.	.	T	T	.	0.82	.	.	F	0.80	2.30
Tyr	139	.	.	.	.	T	T	.	1.38	.	.	F	0.50	1.12
Asn	140	.	.	B	.	.	.	.	0.60	.	.	F	-0.25	0.86
Asn	141	.	.	B	B	.	.	.	0.57	.	.	.	-0.60	0.72
Ile	142	.	.	B	B	.	.	.	-0.13	.	.	.	-0.60	0.45
Ser	143	.	.	B	B	.	.	.	-0.38	.	.	.	-0.60	0.24
Leu	144	.	.	B	B	.	.	.	-0.83	.	.	.	-0.60	0.15
Gly	145	.	.	B	B	.	.	.	-1.53	.	.	.	-0.60	0.19
Phe	146	.	.	B	B	.	.	.	-1.88	.	.	.	-0.60	0.12
Ala	147	.	.	B	B	.	.	.	-1.30	.	.	.	-0.60	0.15
Phe	148	A	.	.	B	.	.	.	-0.96	.	.	.	-0.60	0.21
Phe	149	A	.	.	B	.	.	.	-0.10	.	.	.	-0.26	0.50
Gly	150	A	.	.	.	.	.	.	-0.10	.	.	F	1.33	0.98
Thr	151	.	.	.	.	T	T	.	0.57	.	.	F	2.42	1.12

TABLE III-continued

(PGRP-W)														
Pos.														
Res	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Lys	152	.	.	.	.	T	T	.	0.86	.	.	F	2.76	1.76
Lys	153	.	.	.	.	T	T	.	1.34	.	.	F	3.40	2.39
Gly	154	.	.	.	.	T	T	.	1.74	.	.	F	3.06	2.56
His	155	.	.	.	.	.	.	C	1.88	.	.	F	2.32	1.71
Ser	156	.	.	.	.	.	T	C	1.60	.	.	F	1.88	1.33
Pro	157	.	.	.	.	.	T	C	0.97	.	.	F	0.94	1.35
Ser	158	.	.	.	.	.	T	C	0.11	.	.	F	0.60	1.00
Pro	159	A	.	.	.	.	T	.	0.16	.	.	F	0.25	0.62
Ala	160	A	A	.	.	.	.	.	-0.40	.	.	.	-0.30	0.54
Ala	161	A	A	.	.	.	.	.	-0.70	.	.	.	-0.30	0.40
Leu	162	A	A	.	.	.	.	.	-0.49	.	.	.	-0.60	0.26
Ser	163	A	A	.	.	.	.	.	-0.19	.	.	.	-0.30	0.44
Ala	164	A	A	.	.	.	.	.	-0.79	.	.	.	-0.30	0.71
Met	165	A	A	.	.	.	.	.	-1.09	.	.	.	-0.30	0.71
Glu	166	A	A	.	.	.	.	.	-0.81	.	.	.	-0.30	0.37
Asn	167	A	.	.	B	.	.	.	-0.24	.	.	.	-0.30	0.53
Leu	168	A	.	.	B	.	.	.	-0.53	.	.	.	-0.60	0.83
Ile	169	A	.	.	B	.	.	.	-0.80	.	.	.	-0.60	0.49
Thr	170	A	.	.	B	.	.	.	-0.20	.	.	.	-0.60	0.22
Tyr	171	A	.	.	B	.	.	.	-0.16	.	.	.	-0.60	0.47
Ala	172	A	.	.	B	.	.	.	-0.50	.	.	.	-0.45	1.35
Val	173	A	.	.	B	.	.	.	0.28	.	.	.	-0.30	0.92
Gln	174	.	.	B	.	.	T	.	0.36	.	.	.	0.27	0.80
Lys	175	.	.	B	.	.	T	.	0.37	.	.	F	0.59	0.65
Gly	176	.	.	B	.	.	T	.	0.31	.	.	F	0.91	1.18
His	177	.	.	.	.	.	T	C	0.60	.	.	F	1.73	0.91
Leu	178	.	.	.	.	.	.	C	1.21	.	.	F	1.70	0.61
Ser	179	.	.	.	.	.	T	C	0.36	.	.	F	0.83	0.97
Ser	180	.	.	B	.	.	T	.	0.31	.	.	F	0.46	0.53
Ser	181	.	.	B	.	.	T	.	0.44	.	.	F	0.44	1.11
Tyr	182	.	.	B	.	.	T	.	-0.33	.	.	F	0.57	1.28
Val	183	.	.	B	B	.	.	.	-0.33	.	.	.	-0.60	0.79
Gln	184	.	.	B	B	.	.	.	-0.38	.	.	.	-0.60	0.49
Pro	185	.	.	B	B	.	.	.	-0.03	.	.	F	-0.45	0.31
Leu	186	.	.	B	.	.	.	.	-0.08	.	.	F	0.30	0.83
Leu	187	.	.	B	.	.	.	.	0.17	.	.	F	0.55	0.47
Gly	188	.	.	.	.	T	.	.	1.02	.	.	F	1.80	0.53
Lys	189	.	.	.	.	T	.	.	0.36	.	.	F	2.20	1.03
Gly	190	.	.	.	.	T	T	.	-0.24	.	.	F	2.50	0.67
Glu	191	.	.	.	.	T	T	.	-0.02	.	.	F	2.25	0.56
Asn	192	.	.	B	.	.	T	.	0.58	.	.	F	1.60	0.28
Cys	193	.	.	B	.	.	T	.	1.03	.	.	.	0.60	0.44
Leu	194	.	.	B	.	.	.	.	0.99	.	.	.	0.75	0.50
Ala	195	A	.	.	.	.	T	.	1.38	.	.	.	0.70	0.54
Pro	196	A	.	.	.	.	T	.	1.07	.	.	F	1.00	2.00
Arg	197	A	.	.	.	.	T	.	0.77	.	.	F	1.30	3.51
Gln	198	A	.	.	.	.	T	.	0.62	.	.	F	1.30	4.65
Lys	199	A	A	.	.	.	.	.	1.48	.	.	F	0.90	2.48
Thr	200	A	A	.	.	.	.	.	2.11	.	.	F	0.90	2.53
Ser	201	A	A	.	.	.	.	.	1.51	.	.	F	0.90	2.92
Leu	202	.	A	B	.	.	.	.	0.81	.	.	F	0.90	1.21
Lys	203	.	A	B	.	.	.	.	0.60	.	.	F	0.45	0.84
Lys	204	.	A	B	.	.	.	.	-0.03	.	.	F	0.45	0.97
Leu	205	A	A	.	.	.	.	.	-0.53	.	.	.	0.45	1.19
Ala	206	A	A	.	.	.	.	.	-0.53	.	.	.	0.30	0.49
Pro	207	A	A	.	.	.	.	.	0.24	.	.	.	-0.30	0.33
Ala	208	A	A	.	.	.	.	.	-0.14	.	.	.	-0.60	0.54
Leu	209	A	.	.	.	.	T	.	-1.00	.	.	.	-0.20	0.53
Ser	210	.	.	B	.	.	T	.	-0.86	.	.	.	-0.20	0.28
His	211	.	.	B	.	.	T	.	-0.61	.	.	.	-0.20	0.15
Gly	212	.	.	B	.	.	.	.	-0.40	.	.	.	-0.20	0.18
Leu	213	.	.	B	.	.	.	.	-0.02	.	.	.	0.84	0.23
Cys	214	.	.	.	.	T	.	.	0.44	.	.	.	0.98	0.27
Gly	215	.	.	.	.	T	.	.	0.86	.	.	F	2.07	0.27
Glu	216	.	.	B	.	.	T	.	0.68	.	.	F	2.21	0.63
Pro	217	.	.	.	.	T	T	.	0.21	.	.	F	3.40	1.82
Gly	218	.	.	.	.	T	T	.	0.72	.	.	F	3.06	1.52
Arg	219	.	.	.	.	.	T	C	1.50	.	.	F	2.52	1.17
Pro	220	.	.	.	.	.	.	C	1.24	.	.	F	1.98	1.49
Leu	221	.	.	B	.	.	.	.	0.93	.	.	F	1.14	1.49
Ser	222	.	.	B	.	.	.	.	0.33	.	.	F	0.80	1.10
Arg	223	.	.	B	.	.	.	.	0.47	.	.	.	-0.10	0.58
Met	224	.	.	B	.	.	.	.	-0.23	.	.	.	0.05	1.10

TABLE III-continued

(PGRP-W)														
Pos.														
Res	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Thr	225	.	.	B	.	.	.	.	0.02	.	.	.	-0.10	0.83
Leu	226	.	.	B	.	.	.	.	0.59	.	.	.	0.50	0.84
Pro	227	A	.	.	.	.	.	.	0.54	.	.	.	-0.25	1.34
Ala	228	A	.	.	.	T	.	.	-0.46	.	.	.	0.10	0.92
Lys	229	A	.	.	.	T	.	.	-0.74	.	.	.	-0.20	0.78
Tyr	230	.	.	B	.	T	.	.	-1.32	.	.	.	-0.20	0.35
Gly	231	.	.	B	.	T	.	.	-0.54	.	.	.	-0.20	0.24
Ile	232	.	.	B	B	.	.	.	-0.64	.	.	.	-0.60	0.17
Ile	233	.	.	B	B	.	.	.	-0.64	.	.	.	-0.60	0.15
Ile	234	.	.	B	B	.	.	.	-1.03	.	.	.	-0.60	0.16
His	235	.	.	B	B	.	.	.	-0.68	.	.	.	-0.60	0.22
Thr	236	.	.	B	.	T	.	.	-0.64	.	.	.	0.10	0.62
Ala	237	.	.	.	.	T	T	.	-0.42	.	.	.	0.65	1.27
Gly	238	.	.	.	.	T	T	.	0.47	.	.	F	1.25	0.50
Arg	239	.	.	.	.	T	T	.	0.47	.	.	F	1.25	0.56
Thr	240	.	.	.	B	T	.	.	0.20	.	.	F	0.25	0.39
Cys	241	.	.	.	B	T	.	.	0.51	.	.	.	1.01	0.52
Asn	242	.	.	.	B	T	.	.	1.10	.	.	.	1.32	0.45
Ile	243	.	.	B	B	.	.	.	0.78	.	.	F	1.38	0.54
Ser	244	.	.	B	.	T	.	.	0.78	.	.	F	2.09	0.54
Asp	245	.	.	.	.	T	T	.	0.28	.	.	F	3.10	0.65
Glu	246	A	.	.	.	T	.	.	0.13	.	.	.	2.24	0.77
Cys	247	A	.	.	.	T	.	.	-0.72	.	.	.	1.93	0.47
Arg	248	A	.	.	B	.	.	.	0.28	.	.	.	0.92	0.21
Leu	249	A	.	.	B	.	.	.	0.58	.	.	.	0.61	0.24
Leu	250	A	.	.	B	.	.	.	-0.31	.	.	.	0.30	0.74
Val	251	A	.	.	B	.	.	.	-0.31	.	.	.	0.30	0.27
Arg	252	A	.	.	B	.	.	.	0.06	.	.	.	-0.30	0.56
Asp	253	.	.	B	B	.	.	.	-0.76	.	.	F	0.45	0.91
Ile	254	.	.	B	B	.	.	.	-0.19	.	.	F	0.60	1.06
Gln	255	.	.	B	B	.	.	.	-0.27	.	.	F	-0.15	0.85
Ser	256	.	.	B	B	.	.	.	0.59	.	.	.	-0.60	0.35
Phe	257	.	.	B	B	.	.	.	0.59	.	.	.	-0.60	0.85
Tyr	258	.	.	B	B	.	.	.	-0.22	.	.	.	0.01	0.96
Ile	259	.	.	B	B	.	.	.	0.71	.	.	.	0.32	0.59
Asp	260	.	.	.	B	T	.	.	0.41	.	.	.	1.78	1.36
Arg	261	.	.	.	.	T	.	.	0.04	.	.	F	2.44	1.16
Leu	262	.	.	.	.	T	T	.	0.74	.	.	F	3.10	0.89
Lys	263	.	.	.	.	T	T	.	0.10	.	.	F	2.79	0.89
Ser	264	.	.	B	.	T	.	.	0.64	.	.	F	2.08	0.32
Cys	265	.	.	.	.	T	T	.	0.40	.	.	.	1.72	0.38
Asp	266	.	.	B	.	T	.	.	0.29	.	.	.	1.01	0.30
Ile	267	.	.	B	.	T	.	.	0.40	.	.	.	0.10	0.36
Gly	268	.	.	B	.	T	.	.	-0.46	.	.	.	-0.20	0.58
Tyr	269	.	.	B	.	T	.	.	-1.01	.	.	.	-0.20	0.29
Asn	270	.	.	B	B	.	.	.	-0.69	.	.	.	-0.60	0.30
Phe	271	.	.	B	B	.	.	.	-0.69	.	.	.	-0.60	0.30
Leu	272	.	.	B	B	.	.	.	0.20	.	.	.	-0.60	0.33
Val	273	.	.	B	B	.	.	.	0.20	.	.	.	-0.30	0.35
Gly	274	.	.	B	.	T	.	.	-0.14	.	.	F	0.25	0.40
Gln	275	.	.	B	.	T	.	.	-1.03	.	.	F	0.25	0.49
Asp	276	.	.	.	.	T	.	C	-0.58	.	.	F	0.45	0.46
Gly	277	.	.	.	.	T	.	C	0.23	.	.	F	0.45	0.73
Ala	278	.	.	B	B	.	.	.	0.74	.	.	.	0.30	0.73
Ile	279	.	.	B	B	.	.	.	0.23	.	.	.	0.30	0.43
Tyr	280	.	.	B	B	.	.	.	-0.11	.	.	.	-0.60	0.32
Glu	281	.	.	B	B	.	.	.	-0.40	.	.	.	-0.60	0.32
Gly	282	.	.	.	B	T	.	.	-0.06	.	.	.	-0.20	0.48
Val	283	.	.	B	B	.	.	.	-0.32	.	.	.	-0.60	0.49
Gly	284	.	.	.	B	T	.	.	0.57	.	.	.	-0.20	0.21
Trp	285	.	.	B	B	.	.	.	0.47	.	.	.	-0.60	0.37
Asn	286	.	.	B	B	.	.	.	0.17	.	.	.	-0.60	0.49
Val	287	.	.	B	.	.	T	.	0.21	.	.	F	-0.05	0.66
Gln	288	.	.	B	.	.	T	.	0.76	.	.	F	-0.05	0.84
Gly	289	.	.	.	.	T	T	.	0.89	.	.	F	0.65	0.76
Ser	290	.	.	.	.	T	T	.	0.83	.	.	F	1.14	1.57
Ser	291	.	.	.	.	.	.	C	0.59	.	.	F	1.53	0.90
Thr	292	.	.	.	.	.	T	C	1.44	.	.	F	1.62	1.42
Pro	293	.	.	.	.	.	T	C	1.44	.	.	F	2.56	1.78
Gly	294	.	.	.	.	T	T	.	0.90	.	.	F	3.40	2.21
Tyr	295	.	.	B	.	.	T	.	0.61	.	.	F	2.36	1.07
Asp	296	.	.	A	B	.	.	.	0.10	.	.	F	1.47	0.70
Asp	297	.	.	A	B	B	.	.	0.07	.	.	F	0.53	0.59

TABLE III-continued

(PGRP-W)														
Pos.														
Res	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Ile	298	.	A	B	B	.	.	.	-0.61	.	.	.	0.04	0.37
Ala	299	.	A	B	B	.	.	.	-0.58	.	.	.	0.30	0.16
Leu	300	.	A	B	B	.	.	.	-1.03	.	.	.	-0.60	0.13
Gly	301	.	A	B	B	.	.	.	-1.63	.	.	.	-0.60	0.17
Ile	302	.	.	B	B	.	.	.	-1.98	.	.	.	-0.60	0.16
Thr	303	.	.	B	B	.	.	.	-1.40	.	.	.	-0.60	0.19
Phe	304	.	.	B	B	.	.	.	-1.51	.	.	.	-0.60	0.28
Met	305	.	.	B	B	.	.	.	-1.01	.	.	.	-0.60	0.35
Gly	306	.	.	B	B	.	.	.	-1.01	.	.	.	-0.60	0.35
Thr	307	.	.	B	B	.	.	.	-1.01	.	.	.	-0.60	0.40
Phe	308	.	.	B	B	.	.	.	-0.91	.	.	F	-0.45	0.28
Thr	309	.	.	.	B	T	.	.	-0.42	.	.	F	-0.05	0.44
Gly	310	.	.	.	.	.	.	C	0.18	.	.	F	-0.05	0.48
Ile	311	.	.	.	.	.	.	C	-0.07	.	.	F	-0.05	0.88
Pro	312	.	.	.	.	.	T	C	-0.34	.	.	F	0.45	0.62
Pro	313	.	.	.	.	.	T	C	-0.23	.	.	F	0.45	0.63
Asn	314	.	.	.	.	.	T	C	-0.73	.	.	.	0.00	0.91
Ala	315	A	.	.	.	.	T	.	-0.39	.	.	.	-0.20	0.48
Ala	316	A	A	.	.	.	.	.	-0.09	.	.	.	-0.30	0.54
Ala	317	A	A	.	.	.	.	.	-0.47	.	.	.	-0.30	0.34
Leu	318	A	A	.	.	.	.	.	-0.26	.	.	.	-0.30	0.34
Glu	319	A	A	.	.	.	.	.	-0.26	.	.	.	-0.30	0.58
Ala	320	A	A	.	.	.	.	.	-0.48	.	.	.	0.30	0.97
Ala	321	A	A	.	.	.	.	.	-0.78	.	.	.	0.30	0.97
Gln	322	A	A	.	.	.	.	.	-0.19	.	.	.	0.30	0.39
Asp	323	A	A	.	.	.	.	.	-0.04	.	.	.	-0.30	0.67
Leu	324	A	A	.	.	.	.	.	-0.63	.	.	.	-0.30	0.36
Ile	325	A	A	.	.	.	.	.	-0.64	.	.	.	-0.30	0.21
Gln	326	A	A	.	.	.	.	.	-0.91	.	.	.	-0.60	0.12
Cys	327	A	A	B	.	.	.	.	-0.87	.	.	.	-0.60	0.11
Ala	328	.	A	B	.	.	.	.	-1.21	.	.	.	-0.60	0.32
Met	329	.	A	B	.	.	.	.	-0.64	.	.	.	-0.30	0.18
Val	330	.	A	B	.	.	.	.	-0.57	.	.	.	-0.60	0.53
Lys	331	.	A	B	.	.	.	.	-0.88	.	.	.	-0.60	0.43
Gly	332	.	A	B	.	.	.	.	-0.42	.	.	.	-0.60	0.63
Tyr	333	.	.	B	.	.	.	.	0.17	.	.	.	-0.25	1.31
Leu	334	.	.	B	.	.	.	.	0.52	.	.	F	0.20	1.05
Thr	335	.	.	B	.	T	.	.	0.57	.	.	F	0.10	1.67
Pro	336	.	.	B	.	T	.	.	-0.29	.	.	F	-0.05	0.88
Asn	337	.	.	B	.	T	.	.	-0.80	.	.	.	-0.20	0.88
Tyr	338	.	.	B	.	T	.	.	-0.90	.	.	.	-0.20	0.45
Leu	339	.	.	B	B	.	.	.	-0.12	.	.	.	-0.60	0.29
Leu	340	.	.	B	B	.	.	.	-0.11	.	.	.	-0.60	0.24
Val	341	.	.	B	B	.	.	.	0.10	.	.	.	-0.60	0.21
Gly	342	.	.	B	B	.	.	.	-0.76	.	.	.	-0.30	0.42
His	343	.	.	3	.	.	T	.	-1.10	.	.	.	0.10	0.38
Ser	344	.	.	B	.	.	T	.	-0.18	.	.	.	0.10	0.52
Asp	345	.	.	B	.	.	T	.	0.32	.	F	1.30	1.03	
Val	346	.	.	B	.	.	T	.	0.37	.	.	.	0.85	1.09
Ala	347	.	.	B	B	.	.	.	0.41	.	.	.	0.51	0.67
Arg	348	.	.	B	B	.	.	.	0.23	.	F	0.87	0.54	
Thr	349	.	.	B	B	.	.	.	0.19	.	F	0.63	1.12	
Leu	350	.	.	B	B	.	.	.	0.19	.	F	1.44	1.10	
Ser	351	.	.	.	.	.	T	C	0.46	.	F	2.10	0.97	
Pro	352	.	.	.	.	.	T	C	0.23	.	F	1.29	0.68	
Gly	353	.	.	.	.	T	T	.	-0.12	.	F	0.98	0.68	
Gln	354	.	.	B	.	.	T	.	0.19	.	F	0.37	0.79	
Ala	355	.	.	B	.	.	.	.	0.11	.	.	.	-0.19	0.82
Leu	356	.	.	B	B	.	.	.	-0.48	.	.	.	-0.60	0.56
Tyr	357	.	.	B	B	.	.	.	-0.57	.	.	.	-0.60	0.24
Asn	358	.	.	B	B	.	.	.	-0.53	.	.	.	-0.60	0.31
Ile	359	.	.	B	B	.	.	.	-0.12	.	.	.	-0.60	0.55
Ile	360	.	.	B	B	.	.	.	-0.44	.	.	.	-0.60	0.37
Ser	361	.	.	B	B	.	.	.	0.33	.	.	.	-0.60	0.35
Thr	362	.	.	B	B	.	.	.	-0.12	.	.	.	-0.60	0.69
Trp	363	.	.	.	.	.	T	C	-0.08	.	.	.	0.00	0.85
Pro	364	.	.	.	.	.	T	C	0.78	.	.	.	0.15	1.27
His	365	.	.	.	.	T	T	.	1.28	.	.	.	0.35	1.20
Phe	366	.	.	.	.	T	T	.	1.19	.	.	.	0.35	1.45
Lys	367	.	.	.	.	T	.	.	1.11	.	.	.	0.45	1.20
His	368	.	.	.	.	T	.	.	1.01	.	.	.	0.45	1.13

TABLE IV

(PGRP-C)														
Pos.														
Res	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Met	1	.	.	B	.	.	.	.	0.97	.	.	.	1.64	1.91
Ser	2	.	.	B	.	.	T	.	0.76	.	.	.	2.07	2.00
Arg	3	.	.	B	.	.	T	.	0.33	.	.	.	2.30	1.55
Arg	4	.	.	B	.	.	T	.	-0.09	.	.	.	1.77	1.29
Ser	5	.	.	B	.	.	T	.	-0.29	.	.	.	1.39	0.79
Met	6	.	A	B	.	.	.	.	0.02	.	.	.	0.76	0.41
Leu	7	.	A	B	.	.	.	.	-0.27	.	.	.	-0.37	0.22
Leu	8	.	A	B	.	.	.	.	-1.19	.	.	.	-0.60	0.17
Ala	9	.	A	B	.	.	.	.	-1.51	.	.	.	-0.60	0.14
Trp	10	.	A	B	.	.	.	.	-1.51	.	.	.	-0.60	0.26
Ala	11	.	A	B	.	.	.	.	-1.72	.	.	.	-0.60	0.42
Leu	12	.	.	B	.	T	.	.	-1.72	.	.	.	-0.20	0.34
Pro	13	.	.	B	.	T	.	.	-0.80	.	.	.	-0.20	0.27
Ser	14	.	.	B	.	T	.	.	-1.02	.	.	.	0.10	0.52
Leu	15	.	.	B	.	T	.	.	-1.08	.	.	.	-0.20	0.52
Leu	16	.	A	B	.	.	.	.	-1.08	.	.	.	-0.30	0.33
Arg	17	.	A	B	.	.	.	.	-0.86	.	.	.	-0.30	0.25
Leu	18	.	A	B	.	.	.	.	-0.64	.	.	.	-0.60	0.31
Gly	19	.	A	.	.	.	.	C	-0.34	.	.	.	-0.10	0.65
Ala	20	.	A	.	.	.	.	C	0.16	.	.	.	0.50	0.57
Ala	21	.	A	.	.	.	.	C	0.97	.	.	.	0.65	1.00
Gln	22	.	A	B	.	.	.	.	0.86	.	.	F	1.21	1.75
Glu	23	.	A	B	.	.	.	.	1.46	.	.	F	1.52	2.90
Thr	24	.	A	.	T	.	.	.	1.21	.	.	F	2.23	4.44
Glu	25	.	A	.	T	.	.	.	1.13	.	.	F	2.54	2.59
Asp	26	.	.	.	T	T	.	.	1.06	.	.	F	3.10	0.80
Pro	27	.	.	.	T	T	.	.	0.76	.	.	F	2.49	0.30
Ala	28	.	.	.	T	T	.	.	0.54	.	.	.	2.33	0.23
Cys	29	.	.	.	T	T	.	.	-0.03	.	.	.	1.72	0.21
Cys	30	.	.	B	B	.	.	.	-0.89	.	.	.	-0.29	0.10
Ser	31	.	.	B	B	.	.	.	-1.10	.	.	.	-0.60	0.07
Pro	32	.	.	B	B	.	.	.	-0.78	.	.	.	-0.60	0.20
Ile	33	.	.	B	B	.	.	.	-0.19	.	.	.	0.00	0.75
Val	34	.	.	B	.	T	.	.	0.48	.	.	F	1.45	0.90
Pro	35	.	.	B	.	T	.	.	0.86	.	.	F	1.90	1.01
Arg	36	.	.	.	T	T	.	.	1.20	.	.	F	2.00	1.51
Asn	37	.	.	.	T	T	.	C	0.82	.	.	F	3.00	4.07
Glu	38	.	A	.	T	.	.	.	0.90	.	.	F	2.50	2.66
Trp	39	.	A	.	T	.	.	.	1.17	.	.	F	2.20	1.12
Lys	40	.	A	.	.	.	.	C	1.08	.	.	.	1.10	0.70
Ala	41	.	A	.	.	.	.	C	0.97	.	.	.	0.80	0.54
Leu	42	.	A	.	.	.	.	C	0.30	.	.	.	0.50	0.90
Ala	43	A	A	.	.	.	.	.	-0.29	.	.	.	0.30	0.24
Ser	44	A	A	.	.	.	.	.	0.00	.	.	.	-0.30	0.24
Glu	45	A	A	.	.	.	.	.	-0.08	.	.	.	-0.30	0.50
Cys	46	A	A	.	.	.	.	.	-0.30	.	.	.	0.30	0.68
Ala	47	A	A	.	.	.	.	.	0.21	.	.	.	-0.30	0.42
Gln	48	.	A	B	.	.	.	.	-0.01	.	.	.	-0.30	0.32
His	49	.	A	B	.	.	.	.	0.08	.	.	.	-0.60	0.50
Leu	50	.	A	B	.	.	.	.	-0.73	.	.	.	-0.60	0.76
Ser	51	.	A	B	.	.	.	.	0.04	.	.	.	-0.60	0.36
Leu	52	.	.	B	.	.	.	.	0.39	.	.	.	-0.10	0.52
Pro	53	.	.	B	B	.	.	.	-0.47	.	.	.	-0.60	0.99
Leu	54	.	.	B	B	.	.	.	-1.29	.	.	.	-0.60	0.55
Arg	55	.	.	B	B	.	.	.	-1.33	.	.	.	-0.60	0.49
Tyr	56	.	.	B	B	.	.	.	-1.33	.	.	.	-0.60	0.24
Val	57	.	.	B	B	.	.	.	-0.56	.	.	.	-0.60	0.39
Val	58	.	.	B	B	.	.	.	-0.66	.	.	.	-0.60	0.27
Val	59	.	.	B	B	.	.	.	-0.43	.	.	.	-0.60	0.25
Ser	60	.	.	B	.	.	.	.	-0.89	.	.	.	-0.40	0.34
His	61	.	.	B	.	.	.	.	-0.94	.	.	.	-0.40	0.45
Thr	62	.	.	B	.	.	.	.	-0.39	.	.	.	-0.10	0.81
Ala	63	.	.	.	T	.	.	.	-0.20	.	.	F	0.45	0.81
Gly	64	.	.	.	T	T	.	.	0.66	.	.	F	0.65	0.32
Ser	65	.	.	.	T	T	.	.	0.64	.	.	F	0.65	0.35
Ser	66	.	.	.	T	T	.	.	0.47	.	.	F	0.65	0.51
Cys	67	.	.	.	T	T	.	.	0.19	.	.	F	0.65	0.79
Asn	68	.	.	.	T	.	.	.	0.48	.	.	F	0.45	0.60
Thr	69	.	.	.	.	.	C	.	0.16	.	.	F	0.25	0.60
Pro	70	.	.	.	T	T	.	.	0.46	.	.	F	0.65	0.60
Ala	71	.	.	.	T	T	.	.	0.76	.	.	F	0.65	0.64
Ser	72	.	.	B	.	T	.	.	1.42	.	.	F	0.25	0.77
Cys	73	.	.	B	.	T	.	.	0.83	.	.	F	0.25	0.86

TABLE IV-continued

(PGRP-C)														
Pos.														
Res	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Gln	74	.	A	B	.	.	.	.	1.26	.	.	F	-0.15	0.86
Gln	75	.	A	B	.	.	.	.	1.47	.	.	F	0.60	1.26
Gln	76	.	A	B	.	.	.	.	1.20	.	.	F	0.60	3.79
Ala	77	.	A	B	.	.	.	.	1.50	.	.	F	0.60	1.62
Arg	78	.	A	B	.	.	.	.	2.13	.	.	F	0.60	1.62
Asn	79	.	A	B	.	.	.	.	1.89	.	.	.	0.45	1.28
Val	80	.	A	B	.	.	.	.	1.86	.	.	.	-0.15	1.98
Gln	81	.	A	B	.	.	.	.	1.26	.	.	.	-0.15	1.37
His	82	.	A	B	.	.	.	.	1.89	.	.	.	-0.60	0.85
Tyr	83	.	.	B	.	.	.	.	1.47	.	.	.	-0.25	2.28
His	84	.	.	B	.	.	.	.	0.66	.	.	.	0.05	1.90
Met	85	.	.	B	B	.	.	.	1.17	.	.	.	-0.45	1.15
Lys	86	.	.	B	B	.	.	.	0.88	.	.	.	-0.60	0.73
Thr	87	.	.	.	B	T	.	.	0.24	.	.	.	-0.20	0.56
Leu	88	.	.	.	B	T	.	.	0.49	.	.	.	-0.20	0.30
Gly	89	.	.	.	B	T	.	.	-0.33	.	.	.	0.10	0.25
Trp	90	.	.	B	B	.	.	.	-0.08	.	.	.	-0.60	0.13
Cys	91	.	.	B	B	.	.	.	-0.37	.	.	.	-0.60	0.16
Asp	92	.	.	B	.	T	.	.	-0.06	.	.	.	-0.20	0.25
Val	93	.	.	B	.	T	.	.	0.06	.	.	.	-0.20	0.38
Gly	94	.	.	.	.	T	.	.	-0.41	.	.	.	-0.20	0.61
Tyr	95	.	.	B	.	T	.	.	-1.01	.	.	.	-0.20	0.30
Asn	96	.	.	B	B	.	.	.	-0.69	.	.	.	-0.60	0.29
Phe	97	.	.	B	B	.	.	.	-0.69	.	.	.	-0.60	0.29
Leu	98	.	.	B	B	.	.	.	0.17	.	.	.	-0.60	0.32
Ile	99	.	.	B	B	.	.	.	0.17	.	.	.	0.30	0.33
Gly	100	.	.	B	.	T	.	.	-0.40	.	.	.	0.10	0.38
Glu	101	.	.	B	.	T	.	.	-1.26	.	.	F	0.85	0.38
Asp	102	.	.	.	.	T	T	.	-0.80	.	.	F	1.25	0.40
Gly	103	.	.	.	.	T	.	C	0.01	.	.	F	1.05	0.63
Leu	104	.	.	B	.	.	.	.	0.56	.	.	.	0.50	0.63
Val	105	.	.	B	.	.	.	.	1.01	.	.	.	0.78	0.37
Tyr	106	.	.	B	.	.	.	.	0.67	.	.	.	1.06	0.74
Glu	107	.	.	B	.	.	.	.	0.38	.	.	F	1.49	0.89
Gly	108	.	.	.	.	T	T	.	0.72	.	.	F	1.92	1.25
Arg	109	.	.	.	.	T	T	.	0.83	.	.	F	2.80	1.29
Gly	110	.	.	.	.	T	T	.	1.38	.	.	F	2.37	0.64
Trp	111	.	.	.	.	T	T	.	1.28	.	.	.	1.04	0.94
Asn	112	.	.	.	.	.	.	C	0.69	.	.	.	0.36	0.47
Phe	113	.	.	B	.	.	.	.	1.00	.	.	.	-0.12	0.48
Thr	114	.	.	.	.	.	.	C	0.59	.	.	.	-0.20	0.63
Gly	115	.	.	.	.	.	.	C	0.59	.	.	.	-0.20	0.52
Ala	116	.	.	.	.	.	.	C	0.84	.	.	.	-0.20	0.60
His <sup>b</sup>	117	.	.	.	.	T	.	C	0.03	.	.	.	0.30	0.56
Ser	118	.	.	.	.	T	.	C	0.44	.	.	.	0.00	0.47
Gly	119	.	.	.	.	T	.	C	0.76	.	.	.	0.00	0.49
His	120	.	.	.	.	T	.	C	0.89	.	.	.	0.00	0.58
Leu	121	.	.	.	.	T	.	.	0.88	.	.	.	0.00	0.67
Trp	122	.	.	.	.	.	.	C	0.61	.	.	.	-0.20	0.67
Asn	123	.	.	.	.	.	.	C	0.02	.	.	.	-0.20	0.66
Pro	124	.	.	B	B	.	.	.	0.02	.	.	.	-0.60	0.56
Met	125	.	.	.	B	T	.	.	-0.83	.	.	.	-0.20	0.52
Ser	126	.	.	B	B	.	.	.	-0.32	.	.	.	-0.60	0.23
Ile	127	.	.	B	B	.	.	.	-0.73	.	.	.	-0.60	0.20
Gly	128	.	.	B	B	.	.	.	-1.33	.	.	.	-0.60	0.17
Ile	129	.	.	B	B	.	.	.	-1.47	.	.	.	-0.60	0.13
Ser	130	.	.	B	B	.	.	.	-0.87	.	.	.	-0.60	0.18
Phe	131	.	.	B	B	.	.	.	-0.81	.	.	.	-0.60	0.29
Met	132	.	.	B	.	.	T	.	-0.52	.	.	.	-0.20	0.66
Gly	133	.	.	.	.	T	T	.	-0.18	.	.	.	0.20	0.48
Asn	134	.	.	.	.	T	T	.	0.82	.	.	.	0.20	0.93
Tyr	135	.	.	.	.	T	T	.	0.27	.	.	.	1.25	1.85
Met	136	.	.	.	.	T	.	.	0.76	.	.	.	1.31	1.39
Asp	137	.	.	.	.	T	.	.	1.04	.	.	.	1.57	1.33
Arg	138	.	.	B	.	.	.	.	1.18	.	.	F	1.58	1.23
Val	139	.	.	B	.	.	T	.	1.18	.	.	F	2.04	1.92
Pro	140	.	.	B	.	.	T	.	0.83	.	.	F	2.60	1.99
Thr	141	.	.	.	.	.	T	C	0.54	.	.	F	2.24	1.03
Pro	142	.	.	B	.	.	T	.	0.66	.	.	F	0.73	0.97
Gln	143	.	A	B	.	.	.	.	-0.04	.	.	F	1.12	1.23
Ala	144	.	A	B	.	.	.	.	0.22	.	.	.	0.56	0.86
Ile	145	.	A	B	.	.	.	.	0.43	.	.	.	-0.30	0.56
Arg	146	.	A	B	.	.	.	.	0.40	.	.	.	0.30	0.56

TABLE IV-continued

Res	(PGRP-C)													
	Pos.													
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Ala	147	.	A	B	.	.	.	.	-0.20	*	.	.	-0.30	0.55
Ala	148	.	A	B	.	.	.	.	-1.01	*	.	.	-0.30	0.65
Gln	149	.	A	B	.	.	.	.	-1.01	*	.	.	-0.30	0.27
Gly	150	.	A	B	.	.	.	.	-0.79	*	.	.	-0.60	0.27
Leu	151	.	A	B	.	.	.	.	-1.24	*	.	.	-0.60	0.14
Leu	152	.	A	B	.	.	.	.	-1.51	*	.	.	-0.60	0.08
Ala	153	.	A	B	.	.	.	.	-1.51	*	.	.	-0.60	0.06
Cys	154	.	A	B	.	.	.	.	-1.51	*	.	.	-0.60	0.08
Gly	155	.	A	B	.	.	.	.	-1.51	*	.	.	-0.60	0.16
Val	156	.	A	B	.	.	.	.	-1.29	*	.	.	-0.60	0.16
Ala	157	.	A	B	.	.	.	.	-1.29	*	.	.	-0.60	0.29
Gln	158	.	A	B	.	.	.	.	-0.59	*	.	.	-0.60	0.25
Gly	159	.	A	B	.	.	.	.	-0.22	*	.	.	-0.30	0.65
Ala	160	.	A	B	.	.	.	.	0.12	*	.	F	0.45	0.86
Leu	161	.	A	B	.	.	.	.	0.73	*	.	F	0.45	0.80
Arg	162	.	.	B	.	.	T	.	0.47	*	.	F	0.40	1.26
Ser	163	.	.	B	.	.	T	.	-0.34	*	.	F	0.25	0.93
Asn	164	.	.	B	.	.	T	.	0.04	*	.	F	-0.05	0.93
Tyr	165	.	.	B	.	.	T	.	0.29	*	.	.	0.70	0.95
Val	166	.	.	B	B	.	.	.	1.07	*	.	.	-0.12	0.70
Leu	167	.	.	B	B	.	.	.	1.07	*	.	.	-0.24	0.59
Lys	168	.	.	B	B	.	.	.	1.37	*	.	F	0.39	0.74
Gly	169	.	.	B	.	.	.	.	0.51	*	.	F	1.82	1.67
His	170	.	.	B	B	.	.	.	0.76	*	.	F	1.80	1.50
Arg	171	.	.	B	B	.	.	.	1.72	*	.	F	1.62	1.30
Asp	172	.	.	B	B	.	.	.	2.22	*	.	F	1.44	2.57
Val	173	.	.	B	B	.	.	.	1.37	*	.	F	1.26	2.72
Gln	174	.	.	B	B	.	.	.	1.41	*	.	F	1.08	1.15
Arg	175	.	.	B	B	.	.	.	1.23	*	.	F	0.57	0.92
Thr	176	.	.	B	B	.	.	.	0.78	*	.	F	0.24	1.92
Leu	177	.	.	B	.	.	.	C	0.78	*	.	F	1.16	1.10
Ser	178	.	.	.	.	.	T	C	1.63	*	.	F	1.53	0.90
Pro	179	.	.	.	.	.	T	C	0.82	*	.	F	1.20	1.08
Gly	180	.	.	.	.	T	T	.	0.47	*	.	F	0.98	1.08
Asn	181	.	.	.	.	T	T	.	0.74	*	.	F	0.86	1.26
Gln	182	.	A	B	.	.	.	.	0.74	*	.	F	-0.06	1.11
Leu	183	.	A	B	.	.	.	.	0.16	*	.	.	-0.48	0.93
Tyr	184	.	A	B	.	.	.	.	0.37	*	.	.	-0.60	0.40
His	185	.	A	B	.	.	.	.	0.71	*	.	.	-0.60	0.40
Leu	186	.	A	B	.	.	.	.	0.42	*	.	.	-0.60	0.79
Ile	187	.	A	B	.	.	.	.	0.21	*	.	.	-0.60	0.53
Gln	188	.	A	B	.	.	.	.	0.99	*	.	.	-0.60	0.60
Asn	189	.	A	.	.	T	.	.	0.99	*	.	.	-0.20	0.99
Tyr	190	.	.	.	.	T	.	C	1.13	*	.	.	0.15	2.21
Pro	191	.	.	.	.	T	.	C	1.64	*	.	.	0.45	2.50
His	192	.	.	.	.	T	T	.	2.32	*	.	.	0.86	2.09
Tyr	193	.	.	.	.	T	T	.	1.93	*	.	.	0.77	3.07
Arg	194	.	.	.	.	T	.	.	1.54	*	.	.	1.68	2.54
Ser	195	.	.	.	.	.	.	C	1.44	*	.	.	1.69	2.38
Pro	196	.	.	.	.	T	.	.	1.27	*	.	.	2.10	1.94

Among highly preferred fragments in this regard are those that comprise regions of PGRP-K (SEQ ID NO:2), PGRP-W (SEQ ID NO:4), AND PGRP-C (SEQ ID NO:6) that combine several structural features, such as several of the features set out above.

The polypeptides of the present invention could be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art.

As described in detail below, the polypeptides of the present invention can also be used to raise polyclonal and monoclonal antibodies, which are useful in assays for detecting PGRP-K, PGRP-W, and/or PGRP-C protein expression as described below or as agonists and antagonists capable of enhancing or inhibiting PGRP-K, PGRP-W, and/or PGRP-C protein function. Further, such polypeptides can be used in the yeast two-hybrid system to "capture" PGRP-K, PGRP-W, and/or PGRP-C protein binding pro-

teins which are also candidate agonists and antagonists according to the present invention. The yeast two hybrid system is described in Fields and Song, Nature 340:245-246 (1989).

#### Epitope-Bearing Portions

In another aspect, the invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention. The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known

in the art, for example, by the methods for generating antibodies described *infra* (See, for example, Geysen et al., *Proc. Natl. Acad. Sci. USA* 81:3998-4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross-reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.

Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, *Proc. Natl. Acad. Sci. USA* 82:5131-5135 (1985), further described in U.S. Pat. No. 4,631,211).

In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and, most preferably, between about 15 to about 30 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., *Cell* 37:767-778 (1984); Sutcliffe et al., *Science* 219:660-666 (1983)).

Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., *supra*; Wilson et al., *supra*; Chow et al., *Proc. Natl. Acad. Sci. USA* 82:910-914; and Bittle et al., *J. Gen. Virol.* 66:2347-2354 (1985)). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

Antigenic epitope-bearing peptides and polypeptides of the invention preferably contain a sequence of at least seven, more preferably at least nine and most preferably between about 15 to about 30 amino acids contained within the amino acid sequences of the polypeptides of the invention. Non-limiting examples of antigenic polypeptides or peptides that can be used to generate PGRP-K specific antibodies include: a polypeptide comprising amino acid residues from about Val-24 to about Ala-35 in FIGS. 1A-B (SEQ ID NO:2); a polypeptide comprising amino acid residues from about Gln-51 to about Gln-58 in FIGS. 1A-B (SEQ ID NO:2); a polypeptide comprising amino acid residues from about Gly-69 to about Ser-72 in FIGS. 1A-B (SEQ ID NO:2); a

polypeptide comprising amino acid residues from about Leu-88 to about Gly-100 in FIGS. 1A-B (SEQ ID NO:2); a polypeptide comprising amino acid residues from about His-107 to about Tyr-111 in FIGS. 1A-B (SEQ ID NO:2); a polypeptide comprising amino acid residues from about Gly-122 to about Pro-131 in FIGS. 1A-B (SEQ ID NO:2); a polypeptide comprising amino acid residues from about Gln-146 to about Ile-155 in FIGS. 1A-B (SEQ ID NO:2); a polypeptide comprising amino acid residues from about Leu-159 to about His-170 in FIGS. 1A-B (SEQ ID NO:2); a polypeptide comprising amino acid residues from about Val-172 to about Pro-200 in FIGS. 1A-B (SEQ ID NO:2); a polypeptide comprising amino acid residues from about Gly-211 to about Val-223 in FIGS. 1A-B (SEQ ID NO:2); a polypeptide comprising amino acid residues from about Phe-230 to about Tyr-242 in FIGS. 1A-B (SEQ ID NO:2).

Non-limiting examples of antigenic polypeptides or peptides that can be used to generate PGRP-W specific antibodies include: a polypeptide comprising amino acid residues from about Gly-17 to about Gly-32 in FIGS. 2A-C (SEQ ID NO:4); a polypeptide comprising amino acid residues from about Ile-40 to about Gly-58 in FIGS. 2A-C (SEQ ID NO:4); a polypeptide comprising amino acid residues from about Gly-82 to about Gln-99 in FIGS. 2A-C (SEQ ID NO:4); a polypeptide comprising amino acid residues from about His-104 to about Val-111 in FIGS. 2A-C (SEQ ID NO:4); a polypeptide comprising amino acid residues from about Leu-116 to about Glu-125 in FIGS. 2A-C (SEQ ID NO:4); a polypeptide comprising amino acid residues from about Gly-150 to about Pro-159 in FIGS. 2A-C (SEQ ID NO:4); a polypeptide comprising amino acid residues from about Gln-174 to about Tyr-182 in FIGS. 2A-C (SEQ ID NO:4); a polypeptide comprising amino acid residues from about Leu-186 to about Pro-207 in FIGS. 2A-C (SEQ ID NO:4); a polypeptide comprising amino acid residues from about Val-214 to about Met-225 in FIGS. 2A-C (SEQ ID NO:4); a polypeptide comprising amino acid residues from about Thr-237 to about Val-252 in FIGS. 2A-C (SEQ ID NO:4); a polypeptide comprising amino acid residues from about Tyr-259 to about Ile-268 in FIGS. 2A-C (SEQ ID NO:4); a polypeptide comprising amino acid residues from about Gly-290 to about Ala-300 in FIGS. 2A-C (SEQ ID NO:4); a polypeptide comprising amino acid residues from about His-344 to about Gln-355 in FIGS. 2A-C (SEQ ID NO:4); a polypeptide comprising amino acid residues from about Trp-364 to about His-369 in FIGS. 2A-C (SEQ ID NO:4).

Non-limiting examples of antigenic polypeptides or peptides that can be used to generate PGRP-C specific antibodies include: a polypeptide comprising amino acid residues from about Met-1 to about Met-6 in FIG. 3 (SEQ ID NO:6); a polypeptide comprising amino acid residues from about Ala-20 to about Cys-29 in FIG. 3 (SEQ ID NO:6); a polypeptide comprising amino acid residues from about Ile-33 to about Ala-43 in FIG. 3 (SEQ ID NO:6); a polypeptide comprising amino acid residues from about Ala-63 to about Asn-79 in FIG. 3 (SEQ ID NO:6); a polypeptide comprising amino acid residues from about Ile-99 to about Asn-112 in FIG. 3 (SEQ ID NO:6); a polypeptide comprising amino acid residues from about Gly-133 to about Arg-146 in FIG. 3 (SEQ ID NO:6); a polypeptide comprising amino acid residues from about Ala-160 to about Tyr-165 in FIG. 3 (SEQ ID NO:6); a polypeptide comprising amino acid residues from about Lys-168 to about Asn-181 in FIG. 3 (SEQ ID NO:6); and a polypeptide comprising amino acid residues from about Trp-190 to about Pro-196 in FIG. 3 (SEQ ID NO:6). These polypeptide fragments have been

determined to bear antigenic epitopes of the PGRP-K, PGRP-W, and PGRP-C proteins, respectively, by the analysis of the Jameson-Wolf antigenic index, as shown in FIGS. 6, 8, and 10, above.

Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, in vivo immunization, in vitro immunization, and phage display methods. See, e.g., Sutcliffe et al., *supra*; Wilson et al., *supra*, and Bittle et al., *J. Gen. Virol.*, 66:2347-2354 (1985). If in vivo immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemocyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier-coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 µg of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and as discussed above, the polypeptides of the present invention comprising an immunogenic or antigenic epitope can be fused to other polypeptide sequences. For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof) resulting in chimeric polypeptides. Such fusion proteins may facilitate purification and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., *Nature*, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., *J. Biochem.*, 270:3958-3964 (1995). Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, *Proc. Natl. Acad. Sci. USA* 88:8972-8977). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to

an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni<sup>2+</sup> nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Pat. Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., *Curr. Opinion Biotechnol.* 8:724-33 (1997); Harayama, *Trends Biotechnol.* 16(2): 76-82 (1998); Hansson, et al., *J. Mol. Biol.* 287:265-76 (1999); and Lorenzo and Blasco, *Biotechniques* 24(2): 308-13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety). In one embodiment, alteration of polynucleotides corresponding to SEQ ID NO:X and the polypeptides encoded by these polynucleotides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments by homologous or site-specific recombination to generate variation in the polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of a polynucleotide encoding a polypeptide of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

#### Fusion Proteins

As one of skill in the art will appreciate, PGRP-K, PGRP-W, and/or PGRP-C polypeptides of the present invention and the epitope-bearing fragments thereof described above can be combined with parts of the constant domain of immunoglobulins (agG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EP A 394,827; Traunecker et al., *Nature* 331:84-86 (1988)). Fusion proteins that have a disulfide-linked dimeric structure due to the IgG part can also be more efficient in binding and neutralizing other molecules than the monomeric PGRP-K, PGRP-W, and/or PGRP-C proteins or protein fragments alone (Fountoulakis et al., *J. Biochem.* 270:3958-3964 (1995)).

#### Detection of Disease States

Cells which express either the PGRP-K, PGRP-W and/or PGRP-C polypeptides are believed to have a potent cellular response to infection include, for example, keratinocyte cells, wound-healing tissues, vascular tissues, endothelial tissues, and tissues of the immune, nervous, and endocrine systems. Furthermore, it is believed that cells which express either the PGRP-K, PGRP-W and/or PGRP-C polypeptides regulate apoptosis and/or the proliferation of keratinocytes, epidermal cells, and epithelial cells, as well as mediating the recognition of pathogens (e.g., bacteria) and the processing and presentation of antigens to the immune system. By "a

potent cellular response to infection" is intended any genotypic, phenotypic, and/or morphologic change to a cell, cell line, tissue, tissue culture or patient that is induced by infection from bacterial (Gram positive and negative), viral, fungal, parasitic, etc. As indicated, such cellular responses include not only normal physiological responses infection (e.g., antigenic processing and presentation, immune response), but also diseases associated with aberrant immune system recognition, aberrant antigen processing and presentation in the immune system, aberrant immune system responses to infection, activation, survival, migration and differentiation of immune cells, as well as infections of immuno-compromised individuals, and aberrant regulation of the proliferation/apoptosis of keratinocytes and/or other cells in the body (e.g., immune system cells).

Thus, it is believed that certain tissues in mammals with certain diseases and infections (e.g., bacterial infection of immuno-compromised individuals), diseases associated with increased or decreased cell survival, secretion, activation, migration, differentiation, and proliferation; diseases associated with the defects of wound healing, keratinocyte and cartilage proliferation, cellular immunity, immune dysfunction, and endocrine dysfunction; express significantly altered (e.g., enhanced or decreased) levels of either the PGRP-K, PGRP-W and/or PGRP-C polypeptides and mRNAs encoding the PGRP-K, PGRP-W and/or PGRP-C polypeptides when compared to a corresponding "standard" mammal, i.e., a mammal of the same species not having the disease. Diseases associated with defects in the proliferation of keratinocytes or cartilaginous tissues, include, for example, skin or cartilaginous cancers (such as Chondrosarcomas, basal cell carcinomas, squamous cell carcinomas, melanomas, Chondromatosis, Dyschondroplasia). Diseases associated with immune dysfunction and decreased cellular immunity include, for example, bacterial infections (e.g., cutaneous infection due to *Mycobacterium gordonae* in an AIDS patient) and diseases associated with bacterial infection of the skin (e.g., boils, cellulitis, erysipelas, impetigo).

Further, it is believed that altered levels of either the PGRP-K, PGRP-W and/or PGRP-C polypeptide can be detected in certain body fluids (e.g., lymph, sera, plasma, urine, and spinal fluid) from mammals with the disorder when compared to sera from mammals of the same species not having the disorder. Thus, the invention provides a diagnostic method useful during diagnosis, which involves assaying the expression level of the gene encoding the PGRP-K, PGRP-W and/or PGRP-C polypeptide(s) in mammalian cells or body fluid and comparing the gene expression level with a standard PGRP-K, PGRP-W and/or PGRP-C gene expression level, whereby an increase or decrease in the gene expression level over the standard is indicative of the disease.

By "assaying" the expression level of the gene encoding either the "PGRP-K, PGRP-W and/or PGRP-C polypeptide" is intended qualitatively or quantitatively measuring or estimating the level of the PGRP-K, PGRP-W and/or PGRP-C polypeptide(s) or the level of the mRNA encoding either the PGRP-K, PGRP-W and/or PGRP-C polypeptide(s) in a first biological sample either directly (e.g., by determining or estimating absolute polypeptide or mRNA level) or relatively (e.g., by comparing to either the PGRP-K, PGRP-W and/or PGRP-C polypeptide(s) level or mRNA level in a second biological sample). Preferably, the PGRP-K, PGRP-W and/or PGRP-C protein level or mRNA level in the first biological sample is measured or estimated and compared to a standard PGRP-K, PGRP-W and/or PGRP-C

protein level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disease state. As will be appreciated in the art, once a standard PGRP-K, PGRP-W and/or PGRP-C protein level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, cell line, tissue culture, or other source which contains PGRP-K, PGRP-W and/or PGRP-C protein or mRNA. Biological samples include mammalian body fluids (such as lymph, sera, plasma, urine, synovial fluid and spinal fluid), and keratinocytes, wound-healing tissues, human chondrosarcoma, and other tissues. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source. Where a diagnosis has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting altered PGRP-K, PGRP-W and/or PGRP-C gene expression will experience a worse clinical outcome relative to patients expressing the gene at a normal level.

Nucleic acids for diagnosis may be obtained from a biological sample of a subject, such as from blood, urine, saliva, tissue biopsy or autopsy material, using techniques known in the art. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled PGRP-K, PGRP-W, and/or PGRP-C nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing (see, e.g., Myers et al., *Science* 230:1242 (1985)). Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (see Cotton et al., *Proc. Natl. Acad. Sci. USA* 85:4397-4401 (1988)). In another embodiment, an array of oligonucleotide probes comprising either PGRP-K, PGRP-W, and/or PGRP-C polynucleotide sequences or fragments thereof, can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability (see for example, Chee et al., *Science* 274:610-613 (1996)). The diagnostic assays offer a process for diagnosing or determining a susceptibility to specific diseases through detection of mutations in the PGRP-K, PGRP-W and/or PGRP-C genes by the methods described herein or otherwise known in the art.

In addition, specific diseases can be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of PGRP-K, PGRP-W and/or PGRP-C polypeptides or mRNAs. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art, which include, but are not limited to, Northern blot analysis, (Harada et al., *Cell* 63:303-312 (1990)), S1 nuclease mapping (Fijita et al., *Cell* 49:357-367 (1987)), RNase protection, the polymerase chain reaction (PCR),

reverse transcription in combination with the polymerase chain reaction (RT-PCR) (Makino et al., *Technique* 2:295-301 (1990), reverse transcription in combination with the ligase chain reaction (RT-LCR) and other hybridization methods.

Assaying PGRP-K, PGRP-W, and/or PGRP-C polypeptide levels in a biological sample can be by any techniques known in the art, which include, but are not limited to, radioimmunoassays, competitive-binding assays, Western Blot analysis and enzyme linked immunosorbent assays (ELISAs) and other antibody-based techniques. For example, PGRP-K, PGRP-W, and/or PGRP-C polypeptide expression in tissues can be studied with classical immunohistological methods (Jalkanen et al., *J. Cell. Biol.* 101:976-985 (1985); Jalkanen et al., *J. Cell. Biol.* 105:3087-3096 (1987)). Suitable labels are known in the art and include enzyme labels, such as, Glucose oxidase, and radioisotopes, such as iodine ( $^{125}\text{I}$ ,  $^{131}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{112}\text{In}$ ), and technetium ( $^{99\text{m}}\text{Tc}$ ), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

#### Antibodies

Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment, or variant of SEQ ID NOs: 2, 4, and/or 6, and/or an epitope, of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding). Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')<sub>2</sub>, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, ship rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Pat. No. 5,939,598 by Kucherlapati et al.

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispec-

ficity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., *J. Immunol.* 147:60-69 (1991); U.S. Pat. Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., *J. Immunol.* 148:1547-1553 (1992).

Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or listed in the Tables and Figures. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In specific embodiments, antibodies of the present invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or K<sub>d</sub> less than  $5 \times 10^{-2}$  M,  $10^{-2}$  M,  $5 \times 10^{-3}$  M,  $5 \times 10^{-4}$  M,  $5 \times 10^{-5}$  M,  $5 \times 10^{-6}$  M,  $5 \times 10^{-7}$  M,  $5 \times 10^{-8}$  M,  $5 \times 10^{-9}$  M,  $5 \times 10^{-10}$  M,  $5 \times 10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $5 \times 10^{-14}$  M,  $5 \times 10^{-15}$  M, or  $5 \times 10^{-15}$  M.

The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferably, antibodies of the present invention bind an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described supra). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Pat. No. 5,811,097; Deng et al., *Blood* 92(6):1981-1988 (1998); Chen et al., *Cancer Res.* 58(16):3668-3678 (1998); Harrop et al., *J. Immunol.* 161(4):1786-1794 (1998); Zhu et al., *Cancer Res.* 58(15):3209-3214 (1998); Yoon et al., *J. Immunol.* 160(7):3170-3179 (1998); Prat et al., *J. Cell. Sci.* 111(Pt2):237-247 (1998); Pitard et al., *J. Immunol. Methods* 205(2):177-190 (1997); Liautard et al., *Cytokine* 9(4):233-241 (1997); Carlson et al., *J. Biol. Chem.* 272(17):11295-11301 (1997); Taryman et al., *Neuron* 14(4):755-762 (1995); Muller et al., *Structure* 6(9):1153-1167 (1998); Bartunek et al., *Cytokine* 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N-

or C-terminus or chemically conjugated (including covalently and non-covalently conjugations), to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Pat. No. 5,314,995; and EP 396,387.

The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of-interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (*bacille Calmette-Guerin*) and *corynebacterium parvum*. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art and are discussed in detail in the Examples (e.g., Example 16). In a non-limiting example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for

example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')<sub>2</sub> fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). F(ab')<sub>2</sub> fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., *J. Immunol. Methods* 182:41-50 (1995); Ames et al., *J. Immunol. Methods* 184:177-186 (1995); Kettleborough et al., *Eur. J. Immunol.* 24:952-958 (1994); Persic et al., *Gene* 187 9-18 (1997); Burton et al., *Advances in Immunology* 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Pat. Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')<sub>2</sub> fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., *BioTechniques* 12(6):864-869

(1992); and Sawai et al., *AJRI* 34:26-34 (1995); and Better et al., *Science* 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Pat. Nos. 4,946,778 and 5,258,498; Huston et al., *Methods in Enzymology* 203:46-88 (1991); Shu et al., *PNAS* 90:7995-7999 (1993); and Skerra et al., *Science* 240:1038-1040 (1988). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, *Science* 229:1202 (1985); Oi et al., *BioTechniques* 4:214 (1986); Gillies et al., (1989) *J. Immunol. Methods* 125:191-202; U.S. Pat. Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Pat. No. 5,585,089; Riechmann et al., *Nature* 332:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Pat. Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, *Molecular Immunology* 28(4/5):489-498 (1991); Studnicka et al., *Protein Engineering* 7(6):805-814 (1994); et al., *PNAS* 91:969-973 (1994)), and chain shuffling (U.S. Pat. No. 5,565,332).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Pat. Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the intro-

duction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, *Int. Rev. Immunol.* 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Pat. Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, Calif.) and Genpharm (San Jose, Calif.) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespersen et al., *Bio/technology* 12:899-903 (1988)).

Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, *FASEB J.* 7(5):437-444; (1989) and Nissinoff, *J. Immunol.* 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity. *Polynucleotides Encoding Antibodies*

The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an antibody, preferably, that specifically binds to a polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NOs: 2, 4, and/or 6.

The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method

known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., *Bio-Techniques* 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. and Ausubel et al., eds., 1998, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY, which are both incorporated by reference herein in their entirety), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well known in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., *J. Mol. Biol.* 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778; Bird, Science 242:423-42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* may also be used (Skerra et al., Science 242:1038-1041 (1988)).

#### Methods of Producing Antibodies

The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Pat. No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy

and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., Gene 45:101 (1986); Cockett et al., Bio/Technology 8:2 (1990)).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., EMBO J. 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, Nucleic Acids Res. 13:3101-3109 (1985); Van Heeke & Schuster, J. Biol. Chem. 264:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned indi-

vidually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, *Proc. Natl. Acad. Sci. USA* 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., *Methods in Enzymol.* 153:51-544 (1987)).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., *Cell* 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, *Proc. Natl. Acad. Sci. USA* 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., *Cell* 22:817 (1980)) genes can be employed in tk-, hgprt- or aprt-cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., *Natl. Acad. Sci. USA* 77:357 (1980); O'Hare et al., *Proc. Natl. Acad. Sci. USA* 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, *Proc. Natl. Acad. Sci. USA* 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 *Clinical Pharmacy* 12:488-505; Wu and Wu, *Biotherapy* 3:87-95 (1991); Tolstoshev, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596 (1993); Mulligan, *Science* 260:926-932 (1993); and Morgan and Anderson, *Ann. Rev. Biochem.* 62:191-217 (1993); May, 1993, *TIB TECH* 11(5): 155-215; and hygromycin, which confers resistance to hygromycin (Santerre et al., *Gene* 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1993); Kriegl, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds.), *Current Protocols in Human Genetics*, John Wiley & Sons, NY (1994); Colberre-Garapin et al., *J. Mol. Biol.* 150:1 (1981), which are incorporated by reference herein in their entireties.

The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, *The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning*, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., *Mol. Cell. Biol.* 3:257 (1983)).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, *Nature* 322:52 (1986); Kohler, *Proc. Natl. Acad. Sci. USA* 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition,

the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., supra, and PCT publication WO 93/21232; EP 439,095; Naramura et al., *Immunol. Lett.* 39:91-99 (1994); U.S. Pat. No. 5,474,981; Gillies et al., *PNAS* 89:1428-1432 (1992); Fell et al., *J. Immunol.* 146:2446-2452 (1991), which are incorporated by reference in their entireties.

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Pat. Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., *Proc. Natl. Acad. Sci. USA* 88:10535-10539 (1991); Zheng et al., *J. Immunol.* 154:5590-5600 (1995); and Vil et al., *Proc. Natl. Acad. Sci. USA* 89:11337-11341 (1992) (said references incorporated by reference in their entireties).

As discussed, supra, the polypeptides corresponding to a polypeptide, polypeptide fragment, or a variant of SEQ ID NOs: 2, 4, and/or 6 may be fused or conjugated to the above antibody portions to increase the in vivo half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides corresponding to SEQ ID NOs: 2, 4, and/or 6 may be fused or conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Trautnecker et al., *Nature* 331:84-86 (1988)). The polypeptides of the present invention fused or conjugated to an antibody having disulfide-linked dimeric structures (due to the IgG)

may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., *J. Biochem.* 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP A 232,262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, Bennett et al., *J. Molecular Recognition* 8:52-58 (1995); Johanson et al., *J. Biol. Chem.* 270:9459-9471 (1995)).

Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexahistidine-peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, Calif., 91311), among others, many of which are commercially available. As described in Gentz et al., *Proc. Natl. Acad. Sci. USA* 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., *Cell* 37:767 (1984)) and the "flag" tag.

The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Pat. No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include <sup>125</sup>I, <sup>131</sup>I, <sup>111</sup>In or <sup>99</sup>Tc.

Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g. a cytostatic or cytotoxic agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, <sup>213</sup>Bi. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine,

mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor,  $\alpha$ -interferon,  $\beta$ -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF- $\alpha$ , TNF- $\beta$ , AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi et al., *Int. Immunol.*, 6:1567-1574 (1994)), VEGF (See, International Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Pat. No. 4,676,980, which is incorporated herein by reference in its entirety.

An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

#### Immunophenotyping

The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. The translation product of the gene of the present invention may be useful as a cell specific marker, or more specifically as a cellular marker that is differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Pat. No. 5,985,660; and Morrison et al., *Cell*, 96:737-49 (1999)).

These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

#### Assays For Antibody Binding

The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, *Current Protocols in Molecular Biology*, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasyolol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA; PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 14 hours) at 4° C., adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C., washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, *Current Protocols in Molecular Biology*, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a

polyacrylamide gel (e.g., 8%–20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., <sup>32</sup>P or <sup>125</sup>I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, *Current Protocols in Molecular Biology*, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, *Current Protocols in Molecular Biology*, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., <sup>3</sup>H or <sup>125</sup>I) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with antibody of interest conjugated to a labeled compound (e.g., <sup>3</sup>H or <sup>125</sup>I) in the presence of increasing amounts of an unlabeled second antibody.

#### Therapeutic Uses

The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic

antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives analogs, or nucleic acids, are administered to a human patient for therapy prophylaxis.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention, including fragments thereof. Preferred binding affinities include those with a dissociation constant or K<sub>d</sub> less than 5×10<sup>-2</sup> M, 5×10<sup>-3</sup> M, 5×10<sup>-4</sup> M, 5×10<sup>-5</sup> M, 5×10<sup>-6</sup> M, 5×10<sup>-7</sup> M, 5×10<sup>-8</sup> M, 5×10<sup>-9</sup> M, 5×10<sup>-10</sup> M, 5×10<sup>-11</sup> M, 5×10<sup>-12</sup> M, 5×10<sup>-13</sup> M, 5×10<sup>-14</sup> M, 5×10<sup>-15</sup> M, 10<sup>-15</sup> M. Gene Therapy

In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., *Clinical Pharmacy* 12:488-505 (1993); Wu and Wu, *Biotherapy* 3:87-95 (1991); Tolstoshev, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596 (1993); Mulligan, *Science* 260:926-932 (1993); and Morgan and Anderson, *Ann. Rev. Biochem.* 62:191-217 (1993); May, *TIBTECH* 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1993); and Kriegler, *Gene Transfer and Expression*, A Laboratory Manual, Stockton Press, NY (1990).

In a preferred aspect, the compound comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, *Proc. Natl. Acad. Sci. USA* 86:8932-8935 (1989); Zijlstra et al., *Nature* 342:435-438 (1989)). In specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

In a specific embodiment, the nucleic acid sequences are directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Pat. No. 4,980, 286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, *J. Biol. Chem.* 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recom-

bination (Koller and Smithies, *Proc. Natl. Acad. Sci. USA* 86:8932-8935 (1989); Zijlstra et al., *Nature* 342:435-438 (1989)).

In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., *Meth. Enzymol.* 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., *Biotherapy* 6:291-302 (1994), which describes the use of a retroviral vector to deliver the *mdr1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., *J. Clin. Invest.* 93:644-651 (1994); Kiem et al., *Blood* 83:1467-1473 (1994); Salmons and Gunzberg, *Human Gene Therapy* 4:129-141 (1993); and Grossman and Wilson, *Curr. Opin. in Genetics and Devel.* 3:110-114 (1993).

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, *Current Opinion in Genetics and Development* 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., *Human Gene Therapy* 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., *Science* 252:431-434 (1991); Rosenfeld et al., *Cell* 68:143-155 (1992); Mastrangeli et al., *J. Clin. Invest.* 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., *Gene Therapy* 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., *Proc. Soc. Exp. Biol. Med.* 204:289-300 (1993); U.S. Pat. No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, *Meth. Enzymol.* 217:599-618 (1993); Cohen et al., *Meth. Enzymol.* 217:618-644 (1993); Cline, *Pharmac. Ther.* 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmen-

tal and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, *Cell* 71:973-985 (1992); Rheinwald, *Meth. Cell Bio.* 21A:229 (1980); and Pittelkow and Scott, *Mayo Clinic Proc.* 61:771 (1986)).

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription. Demonstration of Therapeutic or Prophylactic Activity The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

#### Therapeutic/Prophylactic Administration and Composition

The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical composition of the invention, preferably an antibody of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably

an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, *J. Biol. Chem.* 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, *CRC Crit. Rev. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, N.Y. (1984); Ranger and Peppas, *J. Macromol. Sci. Rev. Macromol. Chem.* 23:61 (1983); see also Levy et al., *Science* 228:190 (1985); During et al., *Ann. Neurol.* 25:351 (1989); Howard et al., *J. Neurosurg.* 71:105 (1989)). In yet

another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, supra, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Pat. No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see e.g., Joliot et al., *Proc. Natl. Acad. Sci. USA* 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include

a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

For antibodies, the dosage administered to a patient is typically 0.1 mg/g to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

#### Diagnosis and Imaging

Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases and/or disorders associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific

to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., *J. Cell. Biol.* 101:976-985 (1985); Jalkanen, et al., *J. Cell. Biol.* 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (121In), and technetium (99Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of 99 mTc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S. W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their

Fragments." (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S. W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disorder, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Pat. No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

#### Kits

The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific

embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, Mo.).

The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

#### Formulations

The PGRP-K, PGRP-W, or PGRP-C polypeptide compositions (preferably containing a polypeptide which is a soluble form of the extracellular domain), respectively, will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with either PGRP-K, PGRP-W, or PGRP-C polypeptide alone), the site of delivery of the PGRP-K, PGRP-W, and/or PGRP-C polypeptide composition, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" of PGRP-K, PGRP-W, and/or PGRP-C polypeptide for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of PGRP-K, PGRP-W, and/or PGRP-C polypeptide administered parenterally per dose will be in the range of about 1  $\mu\text{g/kg/day}$  to 10  $\text{mg/kg/day}$  of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01  $\text{mg/kg/day}$ , and most preferably for humans between about 0.01 and 1  $\text{mg/kg/day}$  for the hormone. If given continuously, the PGRP-K, PGRP-W, or PGRP-C polypeptide is typically administered at a dose rate of about 1  $\mu\text{g/kg/hour}$  to about 50  $\mu\text{g/kg/hour}$ , either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Pharmaceutical compositions containing the PGRP-K, PGRP-W, or PGRP-C of the invention may be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), buccally, or as an oral or nasal spray. By "pharmaceutically acceptable carrier" is meant a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

The PGRP-K, PGRP-W and PGRP-C polypeptides are also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules. Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U. et al., *Biopolymers* 22:547-556 (1983)), poly(2-hydroxyethyl methacrylate) (R. Langer et al., *J. Biomed Mater. Res.* 15:167-277 (1981), and R. Langer, *Chem. Tech.* 12:98-105 (1982)), ethylene vinyl acetate (R. Langer et al., Id.) or poly-D-(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release PGRP-K, PGRP-W, and PGRP-C polypeptide compositions also include liposomally entrapped PGRP-K, PGRP-W, and PGRP-C polypeptides. Liposomes containing PGRP-K, PGRP-W, and/or PGRP-C polypeptides are prepared by methods known per se: DE 3,218,121; Epstein et al., *Proc. Natl. Acad. Sci. (USA)* 82:3688-3692 (1985); Hwang et al., *Proc. Natl. Acad. Sci. (USA)* 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal PGRP-K, PGRP-W, or PGRP-C polypeptide therapy.

For parenteral administration, in one embodiment, the PGRP-K, PGRP-W, and PGRP-C polypeptides are formulated generally by mixing them, respectively, at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

Generally, the formulations are prepared by contacting either the PGRP-K, PGRP-W, or PGRP-C polypeptide uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The PGRP-K, PGRP-W, or PGRP-C polypeptide is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of PGRP-K, PGRP-W, or PGRP-C polypeptide salts.

PGRP-K, PGRP-W, or PGRP-C polypeptide to be used for therapeutic administration must be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic PGRP-K, PGRP-W, or PGRP-C polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

PGRP-K, PGRP-W, or PGRP-C polypeptides ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous PGRP-K, PGRP-W, or PGRP-C polypeptide solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized PGRP-K, PGRP-W, or PGRP-C polypeptide using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides of the present invention may be employed in conjunction with other therapeutic compounds.

**Agonists and Antagonists—Assays and Molecules**

The invention also provides a method of screening compounds to identify those which enhance or block the action of PGRP-K, PGRP-W, or PGRP-C on cells, such as its interaction with either PGRP-K, PGRP-W, or PGRP-C bind-

ing molecules such as receptor molecules. An agonist is a compound which increases the natural biological functions of PGRP-K, PGRP-W, or PGRP-C or which functions in a manner similar to PGRP-K, PGRP-W, or PGRP-C while antagonists decrease or eliminate such functions.

In another aspect of this embodiment the invention provides a method for identifying a receptor protein or other ligand-binding protein which binds specifically to a PGRP-K, PGRP-W, or PGRP-C polypeptide. For example, a cellular compartment, such as a membrane or a preparation thereof, may be prepared from a cell that expresses a molecule that binds PGRP-K, PGRP-W, or PGRP-C. The preparation is incubated with labeled PGRP-K, PGRP-W, or PGRP-C and complexes of PGRP-K, PGRP-W, or PGRP-C, respectively, bound to the receptor or other binding proteins are isolated and characterized according to routine methods known in the art. Alternatively, the PGRP-K, PGRP-W, or PGRP-C polypeptide may be bound to a solid support so that binding molecules solubilized from cells are bound to the column and then eluted and characterized according to routine methods.

In the assay of the invention for agonists or antagonists, a cellular compartments, such as a membrane or a preparation thereof, may be prepared from a cell that expresses a molecule that binds PGRP-K, PGRP-W, or PGRP-C such as a molecule of the immune system, such as a macrophage or a monocyte. The preparation is incubated with labeled PGRP-K, PGRP-W, or PGRP-C in the absence or the presence of a candidate molecule which may be a PGRP-K, PGRP-W, or PGRP-C agonist or antagonist. The ability of the candidate molecule to bind the binding molecule is reflected in decreased binding of the labeled ligand. Molecules which bind gratuitously, i.e., without inducing the effects of PGRP-K, PGRP-W, or PGRP-C on binding the PGRP-K, PGRP-W, or PGRP-C binding molecule, are most likely to be good antagonists. Molecules that bind well and elicit effects that are the same as or closely related to PGRP-K, PGRP-W, or PGRP-C are agonists.

PGRP-K, PGRP-W, or PGRP-C-like effects of potential agonists and antagonists may be measured, for instance, by determining activity of a second messenger system following interaction of the candidate molecule with a cell or appropriate cell preparation, and comparing the effect with that of PGRP-K, PGRP-W, or PGRP-C or molecules that elicit the same effects as PGRP-K, PGRP-W, or PGRP-C. Second messenger systems that may be useful in this regard include but are not limited to AMP guanylate cyclase, ion channel or phosphoinositide hydrolysis second messenger systems.

Another example of an assay for PGRP-K, PGRP-W, or PGRP-C antagonists is a competitive assay that combines PGRP-K, PGRP-W, or PGRP-C and a potential antagonist with membrane-bound receptor molecules or recombinant PGRP-K, PGRP-W, or PGRP-C receptor molecules under appropriate conditions for a competitive inhibition assay. PGRP-K, PGRP-W, or PGRP-C can be labeled, such as by radioactivity, such that the number of PGRP-K, PGRP-W, or PGRP-C molecules bound to a receptor molecule can be determined accurately to assess the effectiveness of the potential antagonist.

Potential antagonists include small organic molecules, peptides, polypeptides and antibodies that bind to a polypeptide of the invention and thereby inhibit or extinguish its activity. Potential antagonists also may be small organic molecules, a peptide, a polypeptide, such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a receptor molecule, without inducing

PGRP-K, PGRP-W, or PGRP-C induced activities, thereby preventing the action of PGRP-K, PGRP-W, or PGRP-C by excluding PGRP-K, PGRP-W, or PGRP-C from binding.

Other potential antagonists include antisense molecules. Antisense technology can be used to control gene expression through antisense DNA or RNA or through triple-helix formation. Antisense techniques are discussed, for example, in Okano, J. *Neurochem.* 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, Fla. (1988). Triple helix formation is discussed in, for instance Lee et al., *Nucleic Acids Research* 6: 3073 (1979); Cooney et al., *Science* 241: 456 (1988); and Dervan et al., *Science* 251: 1360 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA. For example, the 5' coding portion of a polynucleotide that encodes the PGRP-like domain of one of the polypeptides of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of PGRP-K, PGRP-W, or PGRP-C, respectively. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into either the PGRP-K, PGRP-W, or PGRP-C polypeptide. The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed in vivo to inhibit production of PGRP-K, PGRP-W, or PGRP-C.

The agonists and antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described above.

The antagonists may be employed for instance to inhibit PGRP-K, PGRP-W, or PGRP-C chemotaxis and activation of macrophages and their precursors, and of neutrophils, monocytes, basophils, B lymphocytes and some T-cell subsets, e.g., activated and CD8 cytotoxic T cells and natural killer cells, in certain auto-immune and chronic inflammatory and infective diseases. Examples of auto-immune diseases include multiple sclerosis, and insulin-dependent diabetes. The antagonists may also be employed to treat infectious diseases including silicosis, sarcoidosis, idiopathic pulmonary fibrosis by preventing the recruitment and activation of mononuclear phagocytes. They may also be employed to treat idiopathic hyper-eosinophilic syndrome by preventing eosinophil production and migration. Endotoxic shock may also be treated by the antagonists by preventing the migration of macrophages and their production of the human chemokine polypeptides of the present invention. The antagonists may also be employed for treating atherosclerosis, by preventing monocyte infiltration in the artery wall. The antagonists may also be employed to treat histamine-mediated allergic reactions and immunological disorders including late phase allergic reactions, chronic urticaria, and atopic dermatitis by inhibiting chemokine-induced mast cell and basophil degranulation and release of histamine. IgE-mediated allergic reactions such as allergic asthma, rhinitis, and eczema may also be treated. The antagonists may also be employed to treat chronic and acute inflammation by preventing the attraction of monocytes to a wound area. They may also be employed to regulate normal pulmonary macrophage populations, since chronic and acute inflammatory pulmonary diseases are associated with sequestration of mononuclear phagocytes in the lung. Antagonists may also be employed to treat rheumatoid arthritis by preventing the attraction of monocytes into synovial fluid in the joints of patients. Monocyte influx and

activation plays a significant role in the pathogenesis of both degenerative and inflammatory arthropathies. The antagonists may be employed to interfere with the deleterious cascades attributed primarily to IL-1 and TNF, which prevents the biosynthesis of other inflammatory cytokines. In this way, the antagonists may be employed to prevent inflammation. The antagonists may also be employed to inhibit prostaglandin-independent fever induced by chemokines. The antagonists may also be employed to treat cases of bone marrow failure, for example, aplastic anemia and myelodysplastic syndrome. The antagonists may also be employed to treat asthma and allergy by preventing eosinophil accumulation in the lung. The antagonists may also be employed to treat subepithelial basement membrane fibrosis which is a prominent feature of the asthmatic lung.

Antibodies against PGRP-K, PGRP-W, or PGRP-C may be employed to bind to and inhibit PGRP-K, PGRP-W, or PGRP-C activity to treat ARDS, by preventing infiltration of neutrophils into the lung after injury. The antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as hereinafter described.

#### Prophylactic and Therapeutic Methods

It is to be understood that although the following discussion is specifically directed to human patients, the teachings are also applicable to any animal that expresses PGRP-K, PGRP-W, or PGRP-C.

PGRP-K, PGRP-W, or PGRP-C polypeptides or polynucleotides (including PGRP-K, PGRP-W, or PGRP-C fragments, variants, derivatives, and analogs, and PGRP-K, PGRP-W, or PGRP-C agonists and antagonists as described herein) are useful to treat or detect infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, PGRP-K, PGRP-W, or PGRP-C polypeptides or polynucleotides and/or PGRP-K, PGRP-W, or PGRP-C agonists or antagonists may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

PGRP-K, PGRP-W, or PGRP-C polypeptides or polynucleotides (including PGRP-K, PGRP-W, or PGRP-C fragments, variants, derivatives, and analogs, and PGRP-K, PGRP-W, or PGRP-C agonists and antagonists as described herein) are useful in treating deficiencies or disorders of the immune system, by activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune deficiencies or disorders may be genetic, somatic, such as cancer or some autoimmune disorders, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, PGRP-K, PGRP-W, or PGRP-C polynucleotides or polypeptides can be used as a marker or detector of a particular immune system disease or disorder.

Similarly, PGRP-K, PGRP-W, or PGRP-C polypeptides or polynucleotides (including PGRP-K, PGRP-W, or PGRP-C fragments, variants, derivatives, and analogs, and PGRP-K, PGRP-W, or PGRP-C agonists and antagonists as described herein) are useful to modulate inflammation. For example, PGRP-K, PGRP-W, or PGRP-C polypeptides or polynucleotides and/or PGRP-K, PGRP-W, or PGRP-C agonists and antagonists of the invention may inhibit the proliferation and differen-

tiation of cells involved in an inflammatory response or alternatively may be involved in killing of hematopoietic cells during processes of inflammation or tissue injury. These molecules can be used to treat inflammatory conditions, both chronic and acute conditions, including ischemia-reperfusion injury, arthritis, and/or nephritis. Additionally, these molecules may be used to treat or prevent killing of hematopoietic cells and/or other cells during processes of inflammation or tissue injury.

PGRP-K, PGRP-W, or PGRP-C polypeptides or polynucleotides (including PGRP-K, PGRP-W, or PGRP-C fragments, variants, derivatives, and analogs, and PGRP-K, PGRP-W, or PGRP-C agonists and antagonists as described herein) are useful to treat or detect hyperproliferative disorders, including neoplasms. PGRP-K, PGRP-W, or PGRP-C polypeptides or polynucleotides and/or PGRP-K, PGRP-W, or PGRP-C agonists or antagonists, may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, PGRP-K, PGRP-W, or PGRP-C polypeptides or polynucleotides and/or PGRP-K, PGRP-W, or PGRP-C agonists or antagonists may proliferate other cells which can inhibit the hyperproliferative disorder. For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative disorders can be treated. This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating hyperproliferative disorders, such as a chemotherapeutic agent.

Given the activities modulated by PGRP-K, PGRP-W, or PGRP-C, it is readily apparent that a substantially altered (increased or decreased) level of expression of PGRP-K, PGRP-W, or PGRP-C in an individual compared to the standard or "normal" level produces pathological conditions such as those described above. It will also be appreciated by one of ordinary skill that the PGRP-K, PGRP-W, or PGRP-C agonists of the invention will exert modulating activities on any of its target cells. Therefore, it will be appreciated that conditions caused by a decrease in the standard or normal level of PGRP-K, PGRP-W, or PGRP-C mediated activity in an individual, can be treated by administration of PGRP-K, PGRP-W, or PGRP-C polypeptide or an agonist thereof.

Thus, in one embodiment, the present invention is directed to a method for enhancing (i.e., increasing) PGRP-K, PGRP-W, or PGRP-C mediated activity (e.g., immunity) which involves administering to an individual in need of an increased level of PGRP-K, PGRP-W, or PGRP-C mediated activity, a therapeutically effective amount of PGRP-K, PGRP-W, or PGRP-C polypeptide, fragment, variant, derivative, or analog, or an agonist capable of increasing PGRP-K, PGRP-W, or PGRP-C mediated activity. In specific embodiments, PGRP-K, PGRP-W, or PGRP-C mediated signaling is increased to treat a disease or condition wherein decreased cell survival, secretion, proliferation, migration, and/or differentiation is exhibited.

In another embodiment, the present invention is directed to a method for suppressing (i.e., decreasing) PGRP-K, PGRP-W, or PGRP-C mediated activity (e.g., inflammation), which involves administering to an individual in need of a decreased level of PGRP-K, PGRP-W, or PGRP-C mediated activity, a therapeutically effective amount of PGRP-K, PGRP-W, or PGRP-C polypeptide, fragment, variant, derivative, or analog or an antagonist capable of decreasing PGRP-K, PGRP-W, or PGRP-C mediated activity. In specific embodiments, PGRP-K, PGRP-W,

or PGRP-C mediated signaling is decreased to treat a disease or condition wherein increased cell survival, secretion, proliferation, migration and/or differentiation is exhibited.

In addition to treating diseases associated with elevated or decreased levels of PGRP-K, PGRP-W, or PGRP-C mediated activity, the invention encompasses methods of administering PGRP-K, PGRP-W, or PGRP-C agonists or antagonists to elevate or reduce PGRP-K, PGRP-W, or PGRP-C mediated biological activity, respectively.

For treating abnormal conditions related to an under-expression of PGRP-K, PGRP-W, or PGRP-C and its activity, or in which elevated or decreased levels of PGRP-K, PGRP-W, or PGRP-C are desired, several approaches are available. One approach comprises administering to an individual in need of an increased level of PGRP-K, PGRP-W, or PGRP-C mediated activity in the body, a therapeutically effective amount of an isolated PGRP-K, PGRP-W, or PGRP-C polypeptide, fragment, variant, derivative or analog of the invention, or a compound which activates PGRP-K, PGRP-W, or PGRP-C, i.e., an agonist as described above, optionally in combination with a pharmaceutically acceptable carrier. Alternatively, gene therapy may be employed to effect the endogenous production of PGRP-K, PGRP-W, or PGRP-C by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector using techniques known in the art. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells in vivo and expression of the polypeptide in vivo. For an overview of gene therapy, see Chapter 20, Gene Therapy and other Molecular Genetic-based Therapeutic Approaches, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996).

Further, treatment can be administered, for example, in the form of gene replacement therapy. Specifically, one or more copies of a PGRP-K, PGRP-W, or PGRP-C nucleotide sequence of the invention that directs the production of a PGRP-K, PGRP-W, or PGRP-C gene product, respectively, exhibiting normal function, may be inserted into the appropriate cells within a patient or animal subject, using vectors which include, but are not limited to, adenovirus, adeno-associated virus, retrovirus and herpesvirus vectors, in addition to other particles that introduce DNA into cells, such as liposomes and gene activated matrices. Because the PGRP-K, PGRP-W, or PGRP-C gene is expressed in epithelial and endothelial tissues, such gene replacement techniques should be capable of delivering PGRP-K, PGRP-W, or PGRP-C gene sequence to these cells within patients, or, alternatively, should involve direct administration of such PGRP-K, PGRP-W, or PGRP-C polynucleotide sequences to the site of the cells in which the PGRP-K, PGRP-W, or PGRP-C gene sequences are to be expressed. Alternatively, targeted homologous recombination can be utilized to correct the defective endogenous PGRP-K, PGRP-W, or PGRP-C gene and/or regulatory sequences thereof (e.g., promoter and enhancer sequences), or alternatively, to "turn on" other dormant PGRP-K, PGRP-W, or PGRP-C activity in the appropriate tissue or cell type.

Additional methods which may be utilized to increase the overall levels of PGRP-K, PGRP-W, or PGRP-C expression and/or PGRP-K, PGRP-W, or PGRP-C activity include the

introduction of appropriate PGRP-K, PGRP-W, or PGRP-C-expressing cells, preferably autologous cells, into a patient at positions and in numbers which are sufficient to ameliorate the symptoms of abnormalities in cell growth regulation, cell signaling, and other PGRP-K, PGRP-W, or PGRP-C mediated activities. Such cells may be either recombinant or non-recombinant. Among the cells which can be administered to increase the overall levels of PGRP-K, PGRP-W, or PGRP-C gene expression in a patient are normal cells, which express the PGRP-K, PGRP-W, or PGRP-C gene. Cell-based gene therapy techniques are well known to those skilled in the art, see, e.g., Anderson et al., U.S. Pat. No. 5,399,349; and Mulligan & Wilson, U.S. Pat. No. 5,460,959.

Thus, one embodiment of the invention comprises administering to an individual in need of an increased level of PGRP-K, PGRP-W, or PGRP-C mediated activity a compound that stimulates PGRP-K, PGRP-W, or PGRP-C mediated activity (agonist), such as for example, an antibody or PGRP-K, PGRP-W, or PGRP-C fragment, variant, derivative or analog of the invention, along with a pharmaceutically acceptable carrier in an amount effective to enhance (i.e., increase) PGRP-K, PGRP-W, or PGRP-C mediated activity.

If the activity of PGRP-K, PGRP-W, or PGRP-C is in excess, several approaches are available to reduce or inhibit PGRP-K, PGRP-W, or PGRP-C activity using molecules derived from the polypeptide and polynucleotide sequences described above. Accordingly, a further aspect of the invention is related to a method for treating an individual in need of a decreased level of PGRP-K, PGRP-W, or PGRP-C mediated activity in the body comprising, administering to such an individual a composition comprising a therapeutically effective amount of a PGRP-K, PGRP-W, or PGRP-C polypeptide, fragment, variant, derivative or analog of the invention which acts as a PGRP-K, PGRP-W, or PGRP-C antagonist or PGRP-K, PGRP-W, or PGRP-C antagonist identified using the methods described herein, optionally, in combination with a pharmaceutically acceptable carrier. Preferably, PGRP-K, PGRP-W, or PGRP-C activity is decreased to treat a disease wherein increased cell survival, secretion, proliferation, migration, and/or differentiation is exhibited. Polypeptides, derivatives, variants and analogs of the invention and other compounds which function as antagonists of PGRP-K, PGRP-W, or PGRP-C can routinely be identified using the assays described infra and other techniques known in the art. Preferred antagonists for use in the present invention are PGRP-K, PGRP-W, or PGRP-C-specific antibodies.

In another approach, PGRP-K, PGRP-W, or PGRP-C activity can be reduced or inhibited by decreasing the level of PGRP-K, PGRP-W, or PGRP-C gene expression, respectively. In one embodiment, this is accomplished through the use of antisense sequences, either internally generated or separately administered (see, for example, O'Connor, J. Neurochem. (1991) 56:560 in *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, Fla. (1988). Antisense technology can be used to control gene expression through antisense DNA or RNA or through triple-helix formation. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56:560 (1991); *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, Fla. (1988). Triple helix formation is discussed in, for instance, Lee et al., *Nucleic Acids Research* 6:3073 (1979); Cooney et al., *Science* 241:456 (1988); and Dervan et al., *Science* 251:1360 (1991). The methods are based on binding of a

polynucleotide to a complementary DNA or RNA. For example, the 5' coding portion of a polynucleotide that encodes a PGRP-K, PGRP-W, or PGRP-C polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the PGRP-K, PGRP-W, or PGRP-C polypeptide. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into polypeptide.

In one embodiment, the PGRP-K, PGRP-W, or PGRP-C antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the PGRP-K, PGRP-W, or PGRP-C antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding PGRP-K, PGRP-W, or PGRP-C, or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bemoist and Chambon, *Nature* 29:304-310 (1981)), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., *Cell* 22:787-797 (1980)), the herpes thymidine promoter (Wagner et al., *Proc. Natl. Acad. Sci. U.S.A.* 78:1441-1445 (1981)), the regulatory sequences of the metallothionein gene (Brinster et al., *Nature* 296:3942 (1982)), etc.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a PGRP-K, PGRP-W, or PGRP-C gene. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double stranded PGRP-K, PGRP-W, or PGRP-C antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid, the more base mismatches with a PGRP-K, PGRP-W, or PGRP-C RNA it may contain and still form a stable duplex (or triplex as the case may be), respectively. One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Potential PGRP-K, PGRP-W, or PGRP-C antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published Oct. 4, 1990; Sarver et al., *Science* 247:1222-1225 (1990)). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy PGRP-K, PGRP-W, or PGRP-C mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and

production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, *Nature* 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within the nucleotide sequences of PGRP-K, PGRP-W, or PGRP-C (FIGS. 1A-C; SEQ ID NO:1). Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of either the PGRP-K, PGRP-W, or PGRP-C mRNA; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. Since ribozymes, unlike antisense molecules are catalytic, a lower intracellular concentration is required for efficiency.

Endogenous PGRP-K, PGRP-W, or PGRP-P gene expression can also be reduced by inactivating or "knocking out" the PGRP-K, PGRP-W, or PGRP-C gene, respectively, or its promoter using targeted homologous recombination (e.g., see Smithies et al., *Nature* 317:330-234 (1985); Thomas et al., *Cell* 51:503-512 (1987); Thompson et al., *Cell* 5:313-321 (1989); each of which is incorporated by reference herein in its entirety). Such approach can be adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site in vivo using appropriate viral vectors.

Alternatively, endogenous PGRP-K, PGRP-W, or PGRP-C gene expression can be reduced by targeted deoxyribonucleotide sequences complementary to the regulatory region of the PGRP-K, PGRP-W, or PGRP-C gene (i.e., the PGRP-K, PGRP-W, or PGRP-C promoters and/or enhancers), respectively, to form triple helical structures that prevent transcription of the PGRP-K, PGRP-W, or PGRP-C genes in target cells in the body, see generally, Helene et al., *Ann. N.Y. Acad. Sci.* 660:27-36 (1992); Helene, C., *Anticancer Drug Des.*, 6(6):569-584 (1991); and Maher, L. J., *Bioassays* 14(12):807-815 (1992)).

Thus, one embodiment of the invention comprises administering to an individual in need of a decreased level of PGRP-K, PGRP-W, or PGRP-C mediated activity, a PGRP-K, PGRP-W, or PGRP-C inhibitor compound (antagonist), such as for example, an antibody or PGRP-K, PGRP-W, or PGRP-C fragment, variant, derivative or analog of the invention, along with a pharmaceutically acceptable carrier in an amount effective to suppress (i.e., lower) PGRP-K, PGRP-W, or PGRP-C mediated activity.

#### Chromosome Assays

The nucleic acid molecules of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

In certain preferred embodiments in this regard, the cDNA herein disclosed is used to clone genomic DNA of a PGRP-K, PGRP-W, and/or PGRP-C protein gene. This can be accomplished using a variety of well known techniques and libraries, which generally are available commercially. The genomic DNA then is used for in situ chromosome mapping using well known techniques for this purpose.

In addition, in some cases, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25

bp) from the cDNA. Computer analysis of the 3' untranslated region of the gene is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Fluorescence in situ hybridization ("FISH") of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with probes from the cDNA as short as 50 or 60 bp. For a review of this technique, see Verma et al., *Human Chromosomes: A Manual Of Basic Techniques*, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, *Mendelian Inheritance In Man*, available on-line through Johns Hopkins University, Welch Medical Library. The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

## EXAMPLES

### Example 1

#### Isolation of PGRP-K, PGRP-W, and/or PGRP-C cDNA Clone(s) From the Deposited Sample(s)

The cDNA for PGRP-K (ATCC Accession No: 203564) is inserted into the Sal I and Not I multiple cloning site of pCMVSPORT 2.0 (Life Technologies). pCMVSPORT 2.0 contains an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, available from Life Technologies. (See, for instance, Gruber, C. E., et al., *Focus* 15:59- (1993).)

The cDNA for PGRP-W (ATCC Accession No: 203563) is inserted into the Sal I and Not I multiple cloning site of pCMVSPORT 3.0 (Life Technologies). pCMVSPORT 3.0 contains an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, available from Life Technologies. (See, for instance, Gruber, C. E., et al., *Focus* 15:59- (1993).)

The cDNA for PGRP-C (ATCC Accession No: 209683) is inserted into the EcoRI and Xho I multiple cloning site of Uni-Zap XR (Stratagene). Uni-Zap XR contains an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, available from Life Technologies. (See, for instance, Gruber, C. E., et al., *Focus* 15:59- (1993).) Two approaches can be used to isolate either PGRP-K, PGRP-W, and/or PGRP-C from the deposited sample. First, a specific polynucleotide of SEQ ID NO:1, SEQ ID NO:3, and SEQ ID NO:5, respectively, with 30-40 nucleotides is synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported. The oligonucleotide is labeled, for instance, with <sup>32</sup>P-g-ATP using T4 polynucleotide kinase and purified according to routine methods. (E.g., Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring, N.Y. (1982).) The plasmid mixture is transformed into a suitable host (such as XL-1

Blue (Stratagene)) using techniques known to those of skill in the art, such as those provided by the vector supplier or in related publications or patents. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate. These plates are screened using Nylon membranes according to routine methods for bacterial colony screening (e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques known to those of skill in the art.

Alternatively, two primers of 17–20 nucleotides derived from both ends of either the SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:5, respectively, (i.e., within the region of SEQ ID NO:1 bounded by the 5' NT and the 3' NT of the clone) are synthesized and used to amplify the PGRP-K, PGRP-W, and/or PGRP-C cDNAs using the deposited cDNA plasmids as template. The polymerase chain reaction is carried out under routine conditions, for instance, in 25 ul of reaction mixture with 0.5 ug of the above cDNA template. A convenient reaction mixture is 1.5–5 mM MgCl<sub>2</sub>, 0.01% (w/v) gelatin, 20 uM each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94 degree C. for 1 min; annealing at 55 degree C. for 1 min; elongation at 72 degree C. for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA product.

Several methods are available for the identification of the 5' or 3' non-coding portions of either the PGRP-K, PGRP-W, or PGRP-C genes which may not be present in the deposited clone. These methods include but are not limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5' and 3' "RACE" protocols which are well known in the art. For instance, a method similar to 5' RACE is available for generating the missing 5' end of a desired full-length transcript. (Fromont-Racine et al., *Nucleic Acids Res.* 21(7):1683–1684 (1993).)

Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of either the PGRP-K, PGRP-W, or PGRP-C gene of interest is used to PCR amplify the 5' portion of the PGRP-K, PGRP-W, or PGRP-C full-length gene, respectively. This amplified product may then be sequenced and used to generate the full length gene.

This above method starts with total RNA isolated from the desired source, although poly-A+ RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer

specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the PGRP-K, PGRP-W, or PGRP-C gene, respectively.

Alternatively, a genomic clone comprising the human PGRP-K, PGRP-W, or PGRP-C coding exons can be isolated by screening a human genomic library as discussed infra. Once positive clones have been identified, the DNA inserts contained in the genomic clone can be isolated, and the DNA sequenced. Once the DNA sequence has been determined, the utilization of a number of computer-based DNA sequence analysis programs, such as, for example, BLAST and GRAIL, will allow the identification of the coding exons and the non-coding introns associated with either the PGRP-K, PGRP-W, or PGRP-C gene, respectively, and hence the identification of any 5' portion of the PGRP-K, PGRP-W, or PGRP-C full-length gene which may not have been previously present in the deposited clone.

#### Example 2

##### Isolation of PGRP-K, PGRP-W, or PGRP-C Genomic Clones

A human genomic P1 library (Genomic Systems, Inc.) is screened by PCR using primers selected for the cDNA sequences corresponding to SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:5, according to the method described in Example 1. (See also, Sambrook.)

#### Example 3

##### Chromosomal Mapping of PGRP-K, PGRP-W, or PGRP-C

An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:5. This primer preferably spans about 100 nucleotides. This primer set is then used in a polymerase chain reaction under the following set of conditions: 30 seconds, 95 degree C.; 1 minute, 56 degree C.; 1 minute, 70 degree C. This cycle is repeated 32 times followed by one 5 minute cycle at 70 degree C. Human, mouse, and hamster DNA is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios, Inc). The reactions is analyzed on either 8% polyacrylamide gels or 3.5% agarose gels. Chromosome mapping is determined by the presence of an approximately 100 bp PCR fragment in the particular somatic cell hybrid.

#### Example 4

##### Bacterial Expression of PGRP-K, PGRP-W, or PGRP-C

PGRP-K, PGRP-W, or PGRP-C polynucleotides encoding PGRP-K, PGRP-W, or PGRP-C polypeptides, respectively, of the invention are amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 1, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites, such as Sal I and Not I, at the 5' end of the primers in order to clone the amplified product into the expression vector. For example, Sal I and Not I correspond to the restriction enzyme sites on the bacterial expression vector pQE-9 (Qiagen, Inc., Chatsworth, Calif.). This plasmid vector encodes antibiotic resistance (Amp<sup>r</sup>), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator

(P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning sites.

To clone either the PGRP-K, PGRP-W, or PGRP-C polypeptide in a bacterial vector, a 5' primer including a restriction site shared by the bacterial vector of interest, and including a number of nucleotides of the amino terminal coding sequence of the sequence of interest, is designed and synthesized. Likewise, a 3' primer including a restriction site shared by the bacterial vector of interest, and including nucleotides complementary to the 3' end of the coding sequence of the sequence of interest, is designed and synthesized. It would be obvious to one skilled in the art as to how to design the primers of interest. The primers are synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported.

For example, specifically, to clone the PGRP-K polypeptide in a bacterial vector, the 5' primer is easily designed by one skilled in the art to clone the PGRP-K polypeptide in a bacterial vector, the 5' primer has the sequence 5' GCA GCACATATGGATTCTCTCGGA ACA AAA CAC AAG CTAAC 3' (SEQ ID NO: 8) containing the underlined NdeI restriction site followed by a number of nucleotides of the amino terminal coding sequence of the full-length PGRP-K sequence in SEQ ID NO:1. One of ordinary skill in the art would appreciate, of course, that the point in the protein coding sequence where the 5' primer begins may be varied to amplify a DNA segment encoding any desired portion of the complete PGRP-K protein shorter or longer than the full-length form of the protein. The 3' primer has the sequence 5' GCAGCAGGTACCTTAGTGTTGAAATG AGGCCAGGTGCTGATGATG 3' (SEQ ID NO: 9) containing the underlined Asp718 restriction site followed by a number of nucleotides complementary to the 3' end of the coding sequence of the PGRP-K DNA sequence of SEQ ID NO:1. PGRP-W and PGRP-C can also be cloned into a bacterial vector using primers and restriction sites specific to those proteins.

The pQE-9 vector is digested with NdeI and Asp718 and the amplified fragment (of either PGRP-K, PGRP-W, or PGRP-C) is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the *E. coli* strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan<sup>r</sup>). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.<sup>600</sup>) of between 0.4 and 0.6. IPTG (Isopropyl-β-D-thiogalactopyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression.

Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000×g).

In addition to the above expression vector, the present invention further includes an expression vector comprising phage operator and promoter elements operatively linked to a PGRP-K, PGRP-W, or PGRP-C polynucleotide, called pHE4a. (ATCC Accession Number 209645, deposited Feb. 25, 1998.) This vector contains: 1) a neomycinphosphotransferase gene as a selection marker, 2) an *E. coli* origin of

replication, 3) a T5 phage promoter sequence, 4) two lac operator sequences, 5) a Shine-Delgarno sequence, and 6) the lactose operon repressor gene (lacI<sub>q</sub>). The origin of replication (oriC) is derived from pUC19 (LTI, Gaithersburg, Md.). The promoter sequence and operator sequences are made synthetically.

DNA can be inserted into the pHE4a by restricting the vector with NdeI and KpnI, BamHI, XhoI, or Asp718, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA insert is generated according to the PCR protocol described in Example 1, using PCR primers having restriction sites for NdeI (5' primer) and XbaI, BamHI, XhoI, or Asp718 (3' primer). The PCR insert is gel purified and restricted with compatible enzymes. The insert and vector are ligated according to standard protocols.

The engineered vector could easily be substituted in the above protocol to express protein in a bacterial system.

#### Example 5

##### Cloning and Expression of PGRP-K, PGRP-W, or PGRP-C in a Baculovirus Expression System

In this example, the plasmid shuttle vector pA2 is used to insert either the PGRP-K, PGRP-W, or PGRP-C polynucleotide into a baculovirus to express PGRP-K, PGRP-W, or PGRP-C, respectively. This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI, XbaI and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak *Drosophila* promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that expresses the cloned PGRP-K, PGRP-W, or PGRP-C polynucleotide, respectively.

Many other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941, and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., *Virol* 170:31-39 (1989).

Specifically, the PGRP-K, PGRP-W, or PGRP-C cDNA sequence contained in the deposited clones, including the AUG initiation codon and any naturally associated leader sequence, is amplified using the PCR protocol described in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the pA2 vector does not need a second signal peptide. Alternatively, the vector can be modified (pA2 GP) to include a baculovirus leader sequence, using the standard methods described in Summers et al., "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin No. 1555 (1987).

To clone either the PGRP-K, PGRP-W, or PGRP-C polypeptide in the baculovirus vector of interest, a 5' primer including a restriction site shared by the baculovirus vector of interest, an efficient signal for initiation of translation in eukaryotic cells (Kozak, M., *J. Mol. Biol.* 196:947-950

(1987)), and including a number of nucleotides of the amino terminal coding sequence of the sequence of interest, is designed and synthesized. Likewise, a 3' primer including a restriction site shared by the bacterial vector of interest, and including nucleotides complementary to the 3' sequence of the sequence of interest, is designed and synthesized. It would be obvious to one skilled in the art as to how to design the primers of interest. The primers are synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported.

For example, specifically, the cDNA sequence encoding the PGRP-K protein in the deposited clone shown in SEQ ID NO:1, is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer has the sequence 5' GCAGCA GGATCCGCCATCATGGGGACGCTGCCATGGCTTCTT GCCTTC 3' (SEQ ID NO: 10) containing the BamHI restriction enzyme site, an efficient signal for initiation of translation in eukaryotic cells (Kozak, M., *J. Mol. Biol.* 196:947-950 (1987)), followed by a number of nucleotides of the sequence of the PGRP-K protein shown in FIGS. 1A-B. The 3' primer has the sequence 5' GCAGCA GGTACCTTATTGATATCCAATGTCACAAAAGTTCCGT GTG 3' (SEQ ID NO: 11) containing the KpnI restriction site followed by a number of nucleotides complementary to the 3' sequence in FIGS. 1A-B.

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Calif.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, Calif.).

The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB 101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, Calif.) cells are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA from individual colonies and analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing.

Five  $\mu$ g of a plasmid containing the polynucleotide is co-transfected with 1.0  $\mu$ g of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, Calif.), using the lipofection method described by Felgner et al., *Proc. Natl. Acad. Sci. USA* 84:7413-7417 (1987). One  $\mu$ g of BaculoGold™ virus DNA and 5  $\mu$ g of the plasmid are mixed in a sterile well of a microtiter plate containing 50  $\mu$ l of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, Md.). Afterwards, 10  $\mu$ l Lipofectin plus 90  $\mu$ l Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27 degrees C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27 degrees C. for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith,

supra. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.) After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200  $\mu$ l of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4 degree C.

To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, Md.). After 42 hours, 5  $\mu$ Ci of  $^{35}$ S-methionine and 5  $\mu$ Ci  $^{35}$ S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the produced PGRP-K, PGRP-W, or PGRP-C polypeptide.

#### Example 6

##### Expression of PGRP-K, PGRP-W, or PGRP-C in Mammalian Cells

PGRP-K, PGRP-W, or PGRP-C polypeptides can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLV, HIV and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2DHFR (ATCC 37146), pBC12MI (ATCC 67109), pCMVSPORT 2.0, and pCMVSPORT 3.0. Mammalian host cells that could be used include, human HeLa, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, PGRP-K, PGRP-W, or PGRP-C polypeptides can be expressed in stable cell lines containing either the PGRP-K, PGRP-W, or PGRP-C polynucleotide integrated into a chromosome, respectively. The cotransfection with a selectable marker such as DHFR, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected PGRP-K, PGRP-W, or PGRP-C gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of interest. (See, e.g., Alt, F. W., et al., *J. Biol. Chem.* 253:1357-1370 (1978); Hamlin, J. L. and, Ma C., *Biochem. et Biophys. Acta*, 1097:107-143 (1990); Page, M. J. and Sydenham M. A., *Biotechnology* 9:64-68 (1991).) Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., *Biochem J.* 227:277-279 (1991); Bebbington et al., *Bio/Technology* 10:169-175 (1992). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

Derivatives of the plasmid pSV2-DHFR (ATCC Accession No. 37146), the expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession No. 209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., *Molecular and Cellular Biology*, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart et al., *Cell* 41:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of either PGRP-K, PGRP-W, or PGRP-C. The vectors also contain the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter.

Specifically, the plasmid pC4 is digested with BamHI and KpnI and then dephosphorylated using calf intestinal phosphatase by procedures known in the art. The vector is then isolated from a 1% agarose gel.

The cDNA sequence encoding either the PGRP-K, PGRP-W, or PGRP-C protein in the respective deposited clone is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer sequence contains an appropriate restriction site, an efficient signal for initiation of translation in eukaryotic cells (Kozak, M., *J. Mol. Biol.* 196:947-950 (1987)), followed by a number of nucleotides of the sequence of the PGRP-K, PGRP-W, or PGRP-C sequence. The 3' primer sequence contains an appropriate restriction site followed by a number of nucleotides complementary to the 3' sequence of the PGRP-K, PGRP-W, or PGRP-C sequence.

For example, specifically, the PGRP-K 5' primer has the sequence 5' GCAGCAGGATCCGCCATCATG GGGACG CTGCCATGGCTTCTTGCCTTC 3' (SEQ ID NO: 12) containing the BamHI restriction enzyme site, an efficient signal for initiation of translation in eukaryotic cells (Kozak, M., *J. Mol. Biol.* 196:947-950 (1987)), followed by a number of nucleotides of the sequence of the PGRP-K protein shown in FIGS. 1A-B. In this embodiment, the 3' primer has the sequence. 5' GCAGCAGGTACCTTATTGATATCCAAT GTCACAAAAGTTCGGTGTG 3' (SEQ ID NO: 13) containing the KpnI restriction site followed by a number of nucleotides complementary to the 3' sequence in FIGS. 1A-B. It would be obvious to one skilled in the art as to how to design the primers of interest. The primers are synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported.

If a naturally occurring signal sequence is used to produce a secreted protein, the vector does not need a second signal peptide. Alternatively, if a naturally occurring signal

sequence is not used, the vector can be modified to include a heterologous signal sequence in an effort to secrete the protein from the cell. (See, e.g., WO 96/34891.)

The amplified fragment is then digested with the BamHI and KpnI and purified on a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Calif.). The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene is used for transfection. Five  $\mu$ g of the expression plasmid pC4 is cotransfected with 0.5  $\mu$ g of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the neo gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MN supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1  $\mu$ M, 2  $\mu$ M, 5  $\mu$ M, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100-200  $\mu$ M. Expression of EITBER PGRP-K, PGRP-W, OR PGRP-C is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

#### Example 7

##### Construction of N-Terminal and/or C-Terminal Deletion Mutants

The following general approach may be used to clone a N-terminal or C-terminal deletion PGRP-K, PGRP-W, or PGRP-C deletion mutant. Generally, two oligonucleotide primers of about 15-25 nucleotides are derived from the desired 5' and 3' positions of a polynucleotide of SEQ ID NO:1. The 5' and 3' positions of the primers are determined based on the desired PGRP-K, PGRP-W, or PGRP-C polynucleotide fragment. An initiation and stop codon are added to the 5' and 3' primers respectively, if necessary, to express the PGRP-K, PGRP-W, or PGRP-C polypeptide fragment encoded by the polynucleotide fragment. Preferred PGRP-K, PGRP-W, or PGRP-C polynucleotide fragments are those encoding the N-terminal and C-terminal deletion mutants disclosed above in the "Polynucleotide and Polypeptide Fragments" section of the Specification.

Additional nucleotides containing restriction sites to facilitate cloning of the PGRP-K, PGRP-W, or PGRP-C polynucleotide fragment in a desired vector may also be added to the 5' and 3' primer sequences. The PGRP-K, PGRP-W, or PGRP-C polynucleotide fragment is amplified from genomic DNA or from the deposited cDNA clone using the appropriate PCR oligonucleotide primers and conditions discussed herein or known in the art. The PGRP-K, PGRP-W, or PGRP-C polypeptide fragments encoded by the PGRP-K, PGRP-W, or PGRP-C polynucleotide fragments, respectively, of the present invention may be expressed and purified in the same general manner as the full

length polypeptides, although routine modifications may be necessary due to the differences in chemical and physical properties between a particular fragment and full length polypeptide.

As a means of exemplifying but not limiting the present invention, the polynucleotide encoding the PGRP-K polypeptide fragment Met-1 to Ile-155 is amplified and cloned as follows: A 5' primer is generated comprising a restriction enzyme site followed by an initiation codon in frame with the polynucleotide sequence encoding the N-terminal portion of the polypeptide fragment beginning with Met-1. A complementary 3' primer is generated comprising a restriction enzyme site followed by a stop codon in frame with the polynucleotide sequence encoding C-terminal portion of the PGRP-K polypeptide fragment ending with Ile-155.

The amplified polynucleotide fragment and the expression vector are digested with restriction enzymes which recognize the sites in the primers. The digested polynucleotides are then ligated together. The PGRP-K polynucleotide fragment is inserted into the restricted expression vector, preferably in a manner which places the PGRP-K polypeptide fragment coding region downstream from the promoter. The ligation mixture is transformed into competent *E. coli* cells using standard procedures and as described in the Examples herein. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis, PCR and DNA sequencing.

#### Example 8

##### Protein Fusions of PGRP-K, PGRP-W, or PGRP-C

PGRP-K, PGRP-W, or PGRP-C polypeptides are preferably fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of PGRP-K, PGRP-W, or PGRP-C polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification. (See Example 5; see also EP A 394,827; Trautner, et al., Nature 331:84-86 (1988).) Similarly, fusion to IgG-1, IgG-3, and albumin increases the half-life time in vivo. Nuclear localization signals fused to PGRP-K, PGRP-W, or PGRP-C polypeptides can target the protein(s) to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule.

Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

For example, if pC4 (Accession No. 209646) is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and the PGRP-K, PGRP-W, or PGRP-C polynucleotide, isolated by the PCR protocol described in Example 1, is ligated into this BamHI site. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced.

If the naturally occurring signal sequence is used to produce the secreted protein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.) Human IgG Fc Region:

```
GGGATCCGGAGCCCCAAATCTTCTGACAAAACCTC
ACACATGCCCCACCGTGCCAGCACCTGAAT
TCGAGGGTGACCGTCAGTCTTCCTCTTCC
CCCCAAAACCCAAGGACACCCTCATGATCT
CCCGGACTCCTGAGGTACATGCGTGGTGG
TGGACGTAAGCCACGAAGACCCTGAGGTCA
AGTTCAACTGGTACGTGGACGGCGTGGAGG
TGCATAATGCCAAGACAAAGCCGCGGGAGG
AGCAGTACAACAGCACGTACCGTGTGGTCA
GCGTCCTCACCGTCCTGCACCAGGACTGGC
TGAATGGCAAGGAGTACAAGTGCAAGGTCT
CCAACAAAGCCCTCCCAACCCCATCGAGA
AAACCATCTCCAAAGCCAAAGGGCAGCCCC
GAGAACCACAGGTGTACACCCTGCCCCAT
CCCGGGATGAGCTGACCAAGAACCAGGTCA
GCCTGACCTGCCTGGTCAAAGGCTTCTATC
CAAGCGACATCGCCGTGGAGTGGGAGAGCA
ATGGGCAGCCGGAGAACAACATAAGACCA
CGCCTCCCGTGCTGGACTCCGACGGCTCCT
TCTTCTCTACAGCAAGCTCACCGTGGACA
AGAGCAGGTGGCAGCAGGGGAACGTCTTCT
CATGCTCCGTGATGCATGAGGCTCTGCACA
ACCACTACACGCAGAAGAGCCTCTCCCTGT
CTCCGGGTAAATGAGTGCGACGGCCGCGAC
TCTAGAGGAT (SEQ ID NO:14)
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#### Example 9

##### Production of an Antibody

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) For example, cells expressing PGRP-K, PGRP-W, or PGRP-C will be administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of PGRP-K, PGRP-W, or PGRP-C protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology. (Köhler et al., Nature 256:495 (1975); Köhler et al., Eur. J. Immunol. 6:511 (1976); Köhler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981).) In general, such procedures involve immunizing an animal (preferably a mouse) with the PGRP-K, PGRP-W, or PGRP-C polypeptide or, more preferably, with a secreted PGRP-K, PGRP-W, or PGRP-C polypeptide-expressing cell. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56 degree C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 ug/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line

may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP20), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the PGRP-K, PGRP-W, or PGRP-C polypeptide, respectively.

Alternatively, additional antibodies capable of binding to the PGRP-K, PGRP-W, or PGRP-C polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the PGRP-K, PGRP-W, or PGRP-C specific antibody can be blocked by PGRP-K, PGRP-W, or PGRP-C, respectively. Such antibodies comprise anti-idiotypic antibodies to the PGRP-K, PGRP-W, or PGRP-C specific antibody and can be used to immunize an animal to induce formation of further PGRP-K, PGRP-W, or PGRP-C specific antibodies, respectively.

It will be appreciated that Fab and F(ab')<sub>2</sub> and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). Alternatively, secreted PGRP-K, PGRP-W, or PGRP-C protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

For in vivo use of antibodies in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., Bio-Techniques 4:214 (1986); Cabilly et al., U.S. Pat. No. 4,816,567; Taniguchi et al., EP.171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

#### Example 10

##### Method of Detecting Abnormal Levels of PGRP-K, PGRP-W, or PGRP-C in a Biological Sample

PGRP-K, PGRP-W, or PGRP-C polypeptides can be detected in a biological sample, and if an increased or decreased level of PGRP-K, PGRP-W, or PGRP-C is detected, the respective polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one skilled in the art can modify the following assay to fit their particular needs.

For example, antibody-sandwich ELISAs are used to detect PGRP-K, PGRP-W, or PGRP-C in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies to PGRP-K, PGRP-W, or

PGRP-C, respectively, at a final concentration of 0.2 to 10 ug/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described in Example 11. The wells are blocked so that non-specific binding of PGRP-K, PGRP-W, or PGRP-C to their respective well is reduced.

The coated wells are then incubated for >2 hours at RT with a sample containing PGRP-K, PGRP-W, or PGRP-C. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbounded PGRP-K, PGRP-W, or PGRP-C.

Next, 50 ul of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbounded conjugate.

Add 75 ul of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot the PGRP-K, PGRP-W, or PGRP-C polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of PGRP-K, PGRP-W, or PGRP-C in the sample using the standard curve.

#### Example 11

##### Formulating a Polypeptide

The PGRP-K, PGRP-W, or PGRP-C composition will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with either the PGRP-K, PGRP-W, or PGRP-C polypeptide alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of PGRP-K, PGRP-W, or PGRP-C administered parenterally per dose will be in the range of about 1 ug/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, PGRP-K, PGRP-W, or PGRP-C is typically administered at a dose rate of about 1 ug/kg/hour to about 50 ug/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Pharmaceutical compositions containing PGRP-K, PGRP-W, or PGRP-C are administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular,

intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

PGRP-K, PGRP-W, or PGRP-C is also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules. Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U. et al., *Biopolymers* 22:547-556 (1983)), poly(2-hydroxyethyl methacrylate) (R. Langer et al., *J. Biomed. Mater. Res.* 15:167-277 (1981), and R. Langer, *Chem. Tech.* 12:98-105 (1982)), ethylene vinyl acetate (R. Langer et al.) or poly-D-(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also include liposomally entrapped PGRP-K, PGRP-W, or PGRP-C polypeptides. Liposomes containing either the PGRP-K, PGRP-W, or PGRP-C are prepared by methods known per se: DE 3,218,121; Epstein et al., *Proc. Natl. Acad. Sci. USA* 82:3688-3692 (1985); Hwang et al., *Proc. Natl. Acad. Sci. USA* 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal secreted polypeptide therapy.

For parenteral administration, in one embodiment, PGRP-K, PGRP-W, or PGRP-C is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

Generally, the formulations are prepared by contacting either PGRP-K, PGRP-W, or PGRP-C uniformly and intimately with liquid carriers or finely divided solid carriers, or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

PGRP-K, PGRP-W, or PGRP-C is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100

mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

PGRP-K, PGRP-W, or PGRP-C used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

PGRP-K, PGRP-W, or PGRP-C polypeptides ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous PGRP-K, PGRP-W, or PGRP-C polypeptide solution, respectively, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized PGRP-K, PGRP-W, or PGRP-C polypeptide using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, PGRP-K, PGRP-W, or PGRP-C may be employed in conjunction with other therapeutic compounds.

#### Example 12

##### Method of Treatment Using Gene Therapy—In Vivo

Another aspect of the present invention is using in vivo gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) PGRP-K, PGRP-W, or PGRP-C sequences into an animal to increase or decrease the expression of the PGRP-K, PGRP-W, or PGRP-C polypeptide, respectively. The PGRP-K, PGRP-W, or PGRP-C polynucleotide may be operatively linked to a promoter or any other genetic elements necessary for the expression of either the PGRP-K, PGRP-W, or PGRP-C polypeptide by the target tissue. Such gene therapy and delivery techniques and methods are known in the art, see, for example, WO90/11092, WO98/11779; U.S. Pat. Nos. 5,693,622, 5,705,151, 5,580,859; Tabata H. et al. (1997) *Cardiovasc. Res.* 35(3):470-479; Chao J et al. (1997) *Pharmacol. Res.* 35(6):517-522; Wolff J. A. (1997) *Neuromuscul. Disord.* 7(5):314-318; Schwartz B. et al. (1996) *Gene Ther.* 3(5):405-411; Tsurumi Y. et al. (1996) *Circulation* 94(12):3281-3290 (incorporated herein by reference).

The PGRP-K, PGRP-W, or PGRP-C polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The PGRP-K, PGRP-W, or PGRP-C polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to

assist, promote, or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the PGRP-K, PGRP-W, or PGRP-C polynucleotides may also be delivered in liposome formulations (such as those taught in Felgner P. L. et al. (1995) Ann. NY Acad. Sci. 772:126-139 and Abdallah B. et al. (1995) Biol. Cell 85(1):1-7) which can be prepared by methods well known to those skilled in the art.

The PGRP-K, PGRP-W, or PGRP-C polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapies techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The PGRP-K, PGRP-W, or PGRP-C polynucleotide constructs can be delivered to the interstitial space of tissues within the an animal, including of pancreas, kidney, muscle, skeletal muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. In vivo muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked PGRP-K, PGRP-W, or PGRP-C polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked PGRP-K, PGRP-W, or PGRP-C polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The dose response effects of injected PGRP-K, PGRP-W, or PGRP-C polynucleotide in muscle in vivo is determined

as follows. Suitable PGRP-K, PGRP-W, or PGRP-C template DNA for production of mRNA coding for PGRP-K, PGRP-W, or PGRP-C polypeptides, respectively, is prepared in accordance with a standard recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. The PGRP-K, PGRP-W, or PGRP-C template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2 cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips.

After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15  $\mu$ m cross-section of the individual quadriceps muscles is histochemically stained for PGRP-K, PGRP-W, or PGRP-C protein expression. A time course for PGRP-K, PGRP-W, or PGRP-C protein expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of PGRP-K, PGRP-W, or PGRP-C DNA in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice will be used to extrapolate proper dosages and other treatment parameters in humans and other animals using PGRP-K, PGRP-W, or PGRP-C naked DNA.

#### Example 13

##### Peptidoglycan Binding Assay

Insoluble peptidoglycan is prepared from *Micrococcus luteus* as has been described in the art (Araki, Y., Nakatani, T., Nakayama, K. and Ito, E., 1972, J. Biol. Chem., 247:6312-632).

The peptidoglycan binding assay is performed by incubating 0.32 mgs of peptidoglycan in 280  $\mu$ ls of 10 mM maleate buffer, pH 6.5/0.15 M NaCl with 3-6  $\mu$ gs of a PGRP in 40  $\mu$ ls of 1 M imidazole/0.5 M NaCl/20 mM Tris-HCl, pH 7.9, for 30 min. at 4° C. One-sixteenth of the supernatant and of the pellet was removed for analysis. The protein was separated for the peptidoglycan by boiling in 2% SDS/PAGE loading buffer, subjected to electrophoresis on an SDS/15% polyacrylamide gel, and stained with Coomassie brilliant blue.

It can be appreciated by those skilled in the art that the above assay may be altered and/or refined to a degree to enhance visualization of the binding, while essentially maintaining the general scheme of the assay. Further peptidoglycan binding assays are described by Yoshida et al., JBC, 271 (23): 13854 (1996), which is incorporated in its entirety by reference herein by reference.

#### Example 14

##### Measurement of Apoptosis Ability of PGRP-K, PGRP-W, or PGRP-C

In a first incubation step, anti-histone antibody is fixed adsorptively on the wall of a microtiter plate module. Subsequently, non-specific binding sites on the wall are

saturated by treatment with incubation buffer (e.g., blocking solution). During the second incubation step, the nucleosomes contained in the appropriate cell (e.g., WEHI 164 cells) sample treated with the PGRP-K, PGRP-W, or PGRP-C bind via their histone components to the immobilized anti-histone antibody. In the third incubation step, anti-DNA-peroxidase reacts with the DNA-part of the nucleosomes. After removal of all unbound peroxidase conjugate by a washing step, the amount of peroxidase retained in the immunocomplex is determined photometrically with ABTS (2,2'-azino-di-[3-ethylbenzthiazoline sulfonate]), as a substrate. Anti-histone antibody reacts with the histones H1, H2A, H2B, H3 and H4 from the sample. Anti-DNA POD antibody binds to single- and double-stranded DNA. Therefore, the ELISA allows the detection on mono- and oligonucleosomes and may be applied to measure apoptotic cell death. The level of cell death is measured by the amount of cytoplasmic histone-associated

DNA fragments which are indicated as the absorbance A405 nm/A490. (See Boehringer mannheim Catalogue, 0990 C 93 2 1541170).

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference.

Moreover, the sequence submitted herewith in paper and computer readable form are herein incorporated by reference in their entireties.

## SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 14

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<211> LENGTH: 1182

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

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ggcctgctga cctgctctgt ggcctacatc atcacagacc agctcccagg gatgcagtgc 240
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&lt;213&gt; ORGANISM: Homo sapiens

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          20             25             30
Pro Leu Ala Cys Arg Ala Leu Leu Thr Leu Pro Val Ala Tyr Ile Ile
      35             40             45
Thr Asp Gln Leu Pro Gly Met Gln Cys Gln Gln Gln Ser Val Cys Ser
  50             55             60
Gln Met Leu Arg Gly Leu Gln Ser His Ser Val Tyr Thr Ile Gly Trp
  65             70             75             80
Cys Asp Val Ala Tyr Asn Phe Leu Val Gly Asp Asp Gly Arg Val Tyr
      85             90             95
Glu Gly Val Gly Trp Asn Ile Gln Gly Leu His Thr Gln Gly Tyr Asn
  100             105             110
Asn Ile Ser Leu Gly Ile Ala Phe Phe Gly Asn Lys Ile Ser Ser Ser
  115             120             125
Pro Ser Pro Ala Ala Leu Ser Ala Ala Glu Gly Leu Ile Ser Tyr Ala
  130             135             140
Ile Gln Lys Gly His Leu Ser Pro Arg Tyr Ile Gln Pro Leu Leu Leu
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Lys Glu Glu Thr Cys Leu Asp Pro Gln His Pro Val Met Pro Arg Lys
  165             170             175
Val Cys Pro Asn Ile Ile Lys Arg Ser Ala Trp Glu Ala Arg Glu Thr
  180             185             190
His Cys Pro Lys Met Asn Leu Pro Ala Lys Tyr Val Ile Ile Ile His
  195             200             205
Thr Ala Gly Thr Ser Cys Thr Val Ser Thr Asp Cys Gln Thr Val Val
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Arg Asn Ile Gln Ser Phe His Met Asp Thr Arg Asn Phe Cys Asp Ile
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&lt;211&gt; LENGTH: 1876

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

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<212> TYPE: PRT  
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

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 20          25          30

Leu Gln Tyr Leu Phe Glu Asn Ile Ser Gln Leu Thr Glu Lys Asp Val
 35          40          45

Ser Thr Thr Val Ser Arg Lys Ala Trp Gly Ala Glu Ala Val Gly Cys
 50          55          60

Ser Ile Gln Leu Thr Thr Pro Val Asn Val Leu Val Ile His His Val
 65          70          75          80

Pro Gly Leu Glu Cys His Asp Gln Thr Val Cys Ser Gln Arg Leu Arg
 85          90          95

Glu Leu Gln Ala His His Val His Asn Asn Ser Gly Cys Asp Val Ala
100          105          110

Tyr Asn Phe Leu Val Gly Asp Asp Gly Arg Val Tyr Glu Gly Val Gly
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Trp Asn Ile Gln Gly Val His Thr Gln Gly Tyr Asn Asn Ile Ser Leu
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 Cys Leu Ala Pro Arg Gln Lys Thr Ser Leu Lys Lys Leu Ala Pro Ala  
 195 200 205  
 Leu Ser His Gly Leu Cys Gly Glu Pro Gly Arg Pro Leu Ser Arg Met  
 210 215 220  
 Thr Leu Pro Ala Lys Tyr Gly Ile Ile Ile His Thr Ala Gly Arg Thr  
 225 230 235 240  
 Cys Asn Ile Ser Asp Glu Cys Arg Leu Leu Val Arg Asp Ile Gln Ser  
 245 250 255  
 Phe Tyr Ile Asp Arg Leu Lys Ser Cys Asp Ile Gly Tyr Asn Phe Leu  
 260 265 270  
 Val Gly Gln Asp Gly Ala Ile Tyr Glu Gly Val Gly Trp Asn Val Gln  
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 Gly Ser Ser Thr Pro Gly Tyr Asp Asp Ile Ala Leu Gly Ile Thr Phe  
 290 295 300  
 Met Gly Thr Phe Thr Gly Ile Pro Pro Asn Ala Ala Ala Leu Glu Ala  
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 Ala Gln Asp Leu Ile Gln Cys Ala Met Val Lys Gly Tyr Leu Thr Pro  
 325 330 335  
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 aaccccatgt ccattggcat cagcttcctg ggcaactaca tggatcgggt gccacacccc 480  
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 <212> TYPE: PRT  
 <213> ORGANISM: Homo sapiens

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Arg Leu Gly Ala Ala Gln Glu Thr Glu Asp Pro Ala Cys Cys Ser Pro
 20           25           30

Ile Val Pro Arg Asn Glu Trp Lys Ala Leu Ala Ser Glu Cys Ala Gln
 35           40           45

His Leu Ser Leu Pro Leu Arg Tyr Val Val Val Ser His Thr Ala Gly
 50           55           60

Ser Ser Cys Asn Thr Pro Ala Ser Cys Gln Gln Gln Ala Arg Asn Val
 65           70           75           80

Gln His Tyr His Met Lys Thr Leu Gly Trp Cys Asp Val Gly Tyr Asn
 85           90           95

Phe Leu Ile Gly Glu Asp Gly Leu Val Tyr Glu Gly Arg Gly Trp Asn
100           105           110

Phe Thr Gly Ala His Ser Gly His Leu Trp Asn Pro Met Ser Ile Gly
115           120           125

Ile Ser Phe Met Gly Asn Tyr Met Asp Arg Val Pro Thr Pro Gln Ala
130           135           140

Ile Arg Ala Ala Gln Gly Leu Leu Ala Cys Gly Val Ala Gln Gly Ala
145           150           155           160

Leu Arg Ser Asn Tyr Val Leu Lys Gly His Arg Asp Val Gln Arg Thr
165           170           175

Leu Ser Pro Gly Asn Gln Leu Tyr His Leu Ile Gln Asn Trp Pro His
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 <212> TYPE: PRT  
 <213> ORGANISM: Homo sapiens

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Cys Ser Phe Ile Val Pro Arg Ser Glu Trp Arg Ala Leu Pro Ser Glu
 20           25           30

Cys Ser Ser Arg Leu Gly His Pro Val Arg Tyr Val Val Ile Ser His
 35           40           45

Thr Ala Gly Ser Phe Cys Asn Ser Pro Asp Ser Cys Glu Gln Gln Ala
 50           55           60

Arg Asn Val Gln His Tyr His Lys Asn Glu Leu Gly Trp Cys Asp Val
 65           70           75           80

Ala Tyr Asn Phe Leu Ile Gly Glu Asp Gly His Val Tyr Glu Gly Arg
 85           90           95

Gly Trp Asn Ile Lys Gly Asp His Thr Gly Pro Ile Trp Asn Pro Met
100           105           110

Ser Ile Gly Ile Thr Phe Met Gly Asn Phe Met Asp Arg Val Arg Lys
115           120           125

Ala Gly Pro Pro Cys Cys Pro Lys Ser Ser Gly Ile Trp Gly Val Ser
130           135           140
  
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<210> SEQ ID NO 13  
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tctcccgagc tcttgaggtc acatgcgtgg tgggtggacgt aagccacgaa gaccctgagg 180

tcaagttcaa ctggtacgtg gacggcgctg aggtgcataa tgccaagaca aagccgcggg 240

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 agaaaacct ctccaaagcc aaagggcagc cccgagaacc acaggtgtac acctgcccc 420  
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 atccaagcga catcgccgtg gagtgggaga gcaatggga gccggagaac aactacaaga 540  
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 acaagagcag gtggcagcag gggaacgtct tctcatgctc cgtgatgat gaggtctgc 660  
 acaaccacta cagcagaag agcctctccc tgtctccggg taaatgagt cgacggcgcc 720  
 gactctagag gat 733

What is claimed is:

1. An isolated protein comprising amino acid residues 52 to 135 of SEQ ID NO:4.

2. The isolated protein of claim 1 which comprises amino acid residues 18 to 368 of SEQ ID NO:4.

3. The isolated protein of claim 1 which comprises amino acid residues 2 to 368 of SEQ ID NO:4.

4. The isolated protein of claim 1 which comprises amino acid residues 1 to 368 of SEQ ID NO:4.

5. The protein of claim 1 which comprises a heterologous polypeptide sequence.

6. A composition comprising the protein of claim 1 and a pharmaceutically acceptable carrier.

7. An isolated protein produced by the method comprising:

- (a) expressing the protein of claim 1 by a cell; and
- (b) recovering said protein.

8. An isolated protein comprising the amino acid sequence of the secreted portion of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 203563.

9. The isolated protein of claim 8 which comprises the amino acid sequence of the complete polypeptide encoded by the cDNA contained in ATCC Deposit No. 203563, excepting the N-terminal methionine.

10. The isolated protein of claim 8 which comprises the amino acid sequence of the complete polypeptide encoded by the cDNA contained in ATCC Deposit No. 203563.

11. The protein of claim 8 which comprises a heterologous polypeptide sequence.

12. A composition comprising the protein of claim 8 and a pharmaceutically acceptable carrier.

13. An isolated protein produced by the method comprising:

- (a) expressing the protein of claim 8 by a cell; and
- (b) recovering said protein.

14. An isolated protein comprising a polypeptide sequence which is at least 90% identical to amino acid residues 18 to 368 of SEQ ID NO:4, wherein said isolated protein possesses peptidoglycan binding activity.

15. The isolated protein of claim 14 wherein said polypeptide sequence is at least 90% identical to amino acid residues 1 to 368 of SEQ ID NO:4.

16. The isolated protein of claim 14 wherein said polypeptide sequence is at least 95% identical to amino acid residues 18 to 368 of SEQ ID NO:4.

17. The isolated protein of claim 14 wherein said polypeptide sequence is at least 95% identical to amino acid residues 1 to 368 of SEQ ID NO:4.

18. The protein of claim 14 which comprises a heterologous polypeptide sequence.

19. A composition comprising the protein of claim 14 and a pharmaceutically acceptable carrier.

20. An isolated protein produced by the method comprising:

- (a) expressing the protein of claim 14 by a cell; and
- (b) recovering said protein.

21. An isolated protein comprising a polypeptide sequence which is at least 90% identical to amino acid residues 52 to 135 of SEQ ID NO:4, wherein said isolated protein possesses peptidoglycan binding activity.

22. The isolated protein of claim 21 wherein said polypeptide sequence is at least 95% identical to amino acid residues 52 to 135 of SEQ ID NO:4.

23. The protein of claim 21 which comprises a heterologous polypeptide sequence.

24. A composition comprising the protein of claim 21 and a pharmaceutically acceptable carrier.

25. An isolated protein produced by the method comprising:

- (a) expressing the protein of claim 21 by a cell; and
- (b) recovering said protein.

26. An isolated protein comprising a polypeptide sequence which is at least 90% identical to the secreted portion of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 203563, wherein said isolated protein possesses peptidoglycan binding activity.

27. The isolated protein of claim 26 wherein said polypeptide sequence is at least 90% identical to the complete polypeptide encoded by the cDNA contained in ATCC Deposit No. 203563.

28. The isolated protein of claim 26 wherein said polypeptide sequence is at least 95% identical to the secreted portion of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 203563.

29. The isolated protein of claim 26 wherein said polypeptide sequence is at least 95% identical to the complete polypeptide encoded by the cDNA contained in ATCC Deposit No. 203563.

30. The protein of claim 26 which comprises a heterologous polypeptide sequence.

31. A composition comprising the protein of claim 26 and a pharmaceutically acceptable carrier.

32. An isolated protein produced by the method comprising:

- (a) expressing the protein of claim 26 by a cell; and
- (b) recovering said protein.

33. An isolated protein comprising at least 30 contiguous amino acid residues of amino acid residues 52 to 135 of SEQ

ID NO:4, wherein said isolated protein possesses peptidoglycan binding activity.

34. The isolated protein of claim 33 which comprises at least 50 contiguous amino acid residues of amino acid residues 52 to 135 of SEQ ID NO:4.

35. The protein of claim 33 which comprises a heterologous polypeptide sequence.

36. A composition comprising the protein of claim 33 and a pharmaceutically acceptable carrier.

37. An isolated protein produced by the method comprising:

- (a) expressing the protein claim 33 by a cell; and
- (b) recovering said protein.

38. An isolated protein comprising at least 30 contiguous amino acid residues of amino acid residues 18 to 368 of SEQ ID NO:4, wherein said isolated protein possesses peptidoglycan binding activity.

39. The isolated protein of claim 38 which comprises at least 50 contiguous amino acid residues of amino acid residues 18 to 368 of SEQ ID NO:4.

40. The protein of claim 38 which comprises a heterologous polypeptide sequence.

41. A composition comprising the protein of claim 38 and a pharmaceutically acceptable carrier.

42. An isolated protein produced by the method comprising:

- (a) expressing the protein of claim 38 by a cell; and
- (b) recovering said protein.

43. An isolated protein comprising at least 30 contiguous amino acid residues of the secreted portion of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 203563, wherein said isolated protein possesses peptidoglycan binding activity.

44. The isolated protein of claim 43 which comprises at least 50 contiguous amino acid residues of the secreted portion of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 203563.

45. The protein of claim 43 which comprises a heterologous polypeptide sequence.

46. A composition comprising the protein of claim 43 and a pharmaceutically acceptable carrier.

47. An isolated protein produced by the method comprising:

- (a) expressing the protein of claim 43 by a cell; and
- (b) recovering said protein.

48. An isolated protein comprising at least 30 contiguous amino acid residues of amino acid residues 1 to 368 of SEQ ID NO:4, wherein said isolated protein possesses peptidoglycan binding activity.

49. The isolated protein of claim 48 which comprises at least 50 contiguous amino acid residues of amino acid residues 1 to 368 of SEQ ID NO:4.

50. The protein of claim 48 which comprises a heterologous polypeptide sequence.

51. A composition comprising the protein of claim 48 and a pharmaceutically acceptable carrier.

52. An isolated protein produced by the method comprising:

- (a) expressing the protein of claim 48 by a cell; and
- (b) recovering said protein.

53. An isolated protein comprising at least 30 contiguous amino acid residues of the complete polypeptide encoded by the cDNA contained in ATCC Deposit No. 203563, wherein said isolated protein possesses peptidoglycan binding activity.

54. The isolated protein of claim 53 which comprises at least 50 contiguous amino acid residues of the complete

polypeptide encoded by the cDNA contained in ATCC Deposit No. 203563.

55. The protein of claim 53 which comprises a heterologous polypeptide sequence.

56. A composition comprising the protein of claim 53 and a pharmaceutically acceptable carrier.

57. An isolated protein produced by the method comprising:

- (a) expressing the protein of claim 53 by a cell; and
- (b) recovering said protein.

58. An isolated protein comprising at least 30 contiguous amino acid residues of amino acid residues 18 to 368 of SEQ ID NO:4, wherein said isolated protein possesses peptidoglycan binding activity.

59. The isolated protein of claim 58 which comprises at least 50 contiguous amino acid residues of amino acid residues 18 to 368 of SEQ ID NO:4.

60. The protein of claim 58 which comprises a heterologous polypeptide sequence.

61. A composition comprising the protein of claim 58 and a pharmaceutically acceptable carrier.

62. An isolated protein produced by the method comprising:

- (a) expressing the protein of claim 58 by a cell; and (b) recovering said protein.

63. An isolated protein comprising a polypeptide sequence selected from the group consisting of:

- (a) amino acid residues 17 to 32 of SEQ ID NO:4;
- (b) amino acid residues 40 to 58 of SEQ ID NO:4;
- (c) amino acid residues 82 to 99 of SEQ ID NO:4;
- (d) amino acid residues 104 to 111 of SEQ ID NO:4;
- (e) amino acid residues 150 to 159 of SEQ ID NO:4;
- (f) amino acid residues 174 to 182 of SEQ ID NO:4;
- (g) amino acid residues 186 to 207 of SEQ ID NO:4;
- (h) amino acid residues 214 to 225 of SEQ ID NO:4;
- (i) amino acid residues 237 to 252 of SEQ ID NO:4;
- (j) amino acid residues 259 to 268 of SEQ ID NO:4;
- (k) amino acid residues 290 to 300 of SEQ ID NO:4; and
- (l) amino acid residues 344 to 355 of SEQ ID NO:4.

64. The isolated protein of claim 63 which comprises polypeptide sequence (a).

65. The isolated protein of claim 63 which comprises polypeptide sequence (b).

66. The isolated protein of claim 63 which comprises polypeptide sequence (c).

67. The isolated protein of claim 63 which comprises polypeptide sequence (d).

68. The isolated protein of claim 67 which further comprises polypeptide sequence (c).

69. The isolated protein of claim 63 which comprises polypeptide sequence (e).

70. The isolated protein of claim 63 which comprises polypeptide sequence (f).

71. The isolated protein of claim 63 which comprises polypeptide sequence (g).

72. The isolated protein of claim 63 which comprises polypeptide sequence (h).

73. The isolated protein of claim 63 which comprises polypeptide sequence (i).

74. The isolated protein of claim 63 which comprises polypeptide sequence (j).

75. The isolated protein of claim 63 which comprises polypeptide sequence (k).

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76. The isolated protein of claim 63 which comprises polypeptide sequence (I).

77. The protein of claim 63 which comprises a heterologous polypeptide sequence.

78. A composition comprising the protein of claim 63 and a pharmaceutically acceptable carrier.

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79. An isolated protein produced by the method comprising:

- (a) expressing the protein of claim 63 by a cell; and
- (b) recovering said protein.

\* \* \* \* \*

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : <b>A61K 38/00, 38/04, 39/12, C07K 5/00, 7/00, 16/00, 17/00</b>		<b>A1</b>	(11) International Publication Number: <b>WO 97/29765</b> (43) International Publication Date: <b>21 August 1997 (21.08.97)</b>
(21) International Application Number: <b>PCT/US97/02218</b> (22) International Filing Date: <b>13 February 1997 (13.02.97)</b> (30) Priority Data: <b>60/011,834</b> <b>16 February 1996 (16.02.96)</b> <b>US</b> (71) Applicant: <b>THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 22nd floor, 300 Lakeside Drive, Oakland, CA 94612-3550 (US).</b> (72) Inventor: <b>SELSTED, Michael, E.; 16 Young Court, Irvine, CA 92715 (US).</b> (74) Agent: <b>BERLINER, Robert; Robbins, Berliner &amp; Carson, 5th floor, 201 N. Figueroa Street, Los Angeles, CA 90012-2628 (US).</b>		(81) Designated States: <b>AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</b>  <b>Published</b> <i>With international search report.</i>	
(54) Title: <b>ANTIMICROBIAL PEPTIDES AND METHODS OF USE</b>			
(57) Abstract <p>Novel antimicrobial peptides from bovine and murine neutrophils are provided. The peptides, designated bovine granulocyte peptide A (BGP-A) and murine granulocyte peptide A (MGP-A) were purified to homogeneity from peripheral blood granulocytes. The amino acid and nucleotide sequence of BGP-A and MGP-A are also provided. A synthetic version of BGP-A and MGP-A is also provided. The purified BGP-A peptide is shown to have antimicrobial activity indistinguishable from that of natural BGP-A. Synthetic carboxamidated analogs of BGP-A (BGP-A-amide) and MGP-A (MGP-A-amide) are also provided.</p>			

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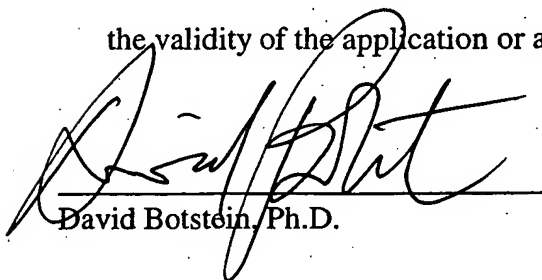
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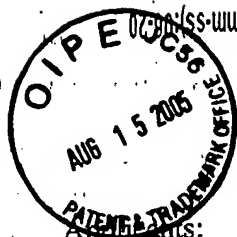
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Audrey Goddard, Ph.D.

*10/6/04*  
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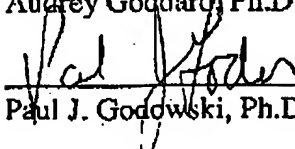
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11/21/04  
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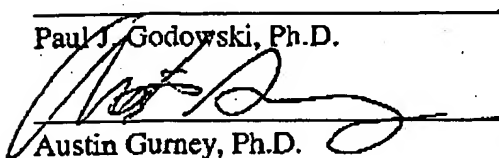
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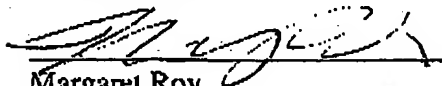
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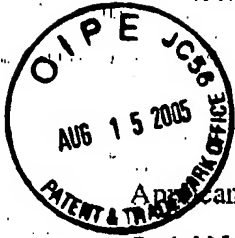
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David Botstein, Ph.D.

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Date

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Audrey Goddard, Ph.D.

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Date

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Paul J. Godowski, Ph.D.

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Austin Gurney, Ph.D.

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Margaret Roy

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Date

Colin K. Watanabe  
Colin K. Watanabe

Oct 8, 2004  
Date

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William I. Wood, Ph.D.

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Date

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10/5/04 8:54 AM (39780.2830)



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Baker et al. Docket No: 39780-2880P1C53  
Serial No: 10/015,390 Group Art Unit: 1637  
Filed: December 12, 2001 Examiner: Fredman, Jeffrey N.  
For: **SECRETED AND TRANSMEMBRANE POLYPEPTIDES AND NUCLEIC  
ACIDS ENCODING THE SAME**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, Virginia 22313-1450

**DECLARATION OF DAVID BOTSTEIN, Ph.D.,**  
**AUDREY GODDARD, Ph.D., PAUL J. GODOWSKI, Ph.D.,**  
**AUSTIN GURNEY, Ph.D., MARGARET ROY, COLIN K. WATANABE and**  
**WILLIAM I. WOOD, Ph.D. UNDER 37 CFR 1.131**

We, David Botstein, Ph.D., Audrey Goddard, Ph.D., Paul J. Godowski, Ph.D., Austin Gurney, Ph.D., Margaret Roy, Colin K. Watanabe and William I. Wood, Ph.D. declare and say as follows:

1. We are the inventors of the above-identified application.
2. We have read and understood the claims pending in this application, and are aware that the claims have been rejected as anticipated by U.S. Patent No. 6,444,790 (Young *et al.*, issue date September 3, 2002 and effective priority date December 23, 1998).
3. The polypeptide designated as PRO1269 (SEQ ID NO:216) claimed in the above-identified application in the United States was sequenced, cloned and homology to granulocyte peptide A identified prior to December 23, 1998.
4. U.S. Provisional Application No. 60/100,661, filed on September 16, 1998 discloses sequences designated as SEQ ID NO:2 and SEQ ID NO:1, which are identical to SEQ ID NO:215 and SEQ ID NO:216, respectively, of the above-identified application.

5. U.S. Provisional Application No. 60/100,661, filed on September 16, 1998 further discloses that SEQ ID NO:1, corresponding to SEQ ID NO: 216 of the above-identified application, has homology to granulocyte peptide A.
6. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information or belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

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David Botstein, Ph.D.

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Audrey Goddard, Ph.D.

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Paul J. Godowski, Ph.D.

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Austin Gurney, Ph.D.

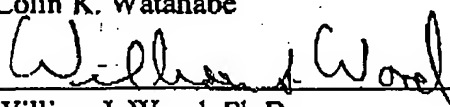
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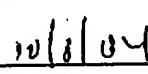
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## ANTIMICROBIAL PEPTIDES AND METHODS OF USE

This invention was made with Government support under Grant No. AI22931 awarded by the National Institutes of Health. The Government has certain rights in this invention.

### 5 1. *Field of the Invention*

This invention relates generally to antimicrobial peptides, and, more specifically, to peptides designated bovine granulocyte peptide -A (BGP-A), bovine granulocyte peptide -A-amide (BGP-A-amide), murine granulocyte peptide -A(MGP-A) and murine granulocyte peptide -A-amide (MGP-A-amide) and methods of uses thereof.

### 10 2. *Background of the Invention*

The cytoplasmic granules of polymorphonuclear leukocytes (neutrophils, granulocytes, PMNs) contain antimicrobial peptides that allow these cells to inactivate ingested microbial targets by mechanisms considered "oxygen independent" (Lehrer, R. I., *et al.*, *Blood* 76:2169-2181, 1990). These granule proteins constitute an antimicrobi-  
15 al arsenal that includes defensins (Selsted, M.E., *et al.*, *Trends in Cell Biology* 5:114-119, 1995),  $\beta$ -defensins (Selsted, M.E., *et al.*, *J. Biol. Chem.* 268:6641-6648, 1993), indolicidin (Selsted, M.E., *et al.*, *J. Biol. Chem.* 267:4292-4295, 1992), and other broad spectrum antibiotic peptides that are released into the phagosome during phagolysosome fusion. To date, members of the defensin family have been isolated from neutrophils  
20 of human (Ganz, T., *et al.*, *J. Clin. Invest.* 76:1427-1435, 1985), rabbit (Selsted, M.E., *et al.*, *J. Biol. Chem.* 260:4579-4584, 1985), rat (Eisenhauer, P., *et al.*, *Immun.* 58:3899-3902, 1990), and guinea pig origin (Selsted, M.E., *et al.*, *Infect. Immun.* 55:2281-2286, 1987), and most recently from the Paneth cells of mouse small intestine (Selsted, M.E., *et al.*, *J. Cell Biol.* 118:929-936, 1992).  $\beta$ -defensins have been isolated from the large  
25 granules of bovine neutrophils (Selsted, M.E., *et al.*, *J. Biol. Chem.* 268:6641-6648, 1993), bovine tracheal epithelium (Diamond, G.M., *et al.*, *Proc. Natl. Acad. Sci. USA* 88:3952-3956, 1991), and human plasma (Bensch, K. W., *et al.*, *FEBS Lett.* 368:331-

-2-

335), and indolicidin is a component of the large granules of bovine PMN (Van Abel, R.J., *et al.*, *Int. J. Peptide Protein* 45:401-409, 1995).

The unique features of ruminant granulocytes were first described by Gennaro and Baggiolini and coworkers (Baggiolini, M., *et al.*, *Lab. Invest.* 52:151-158, 1985; 5 Gennaro, R., *et al.*, *J. Cell Biol.* 96:1651-1661, 1983) who demonstrated that neutrophils of cattle, goats, sheep, and ibex are endowed with many unusually large cytoplasmic granules that are distinct from the classical azurophil and specific granules. Subsequent studies established that most of the antibacterial peptides of bovine neutrophils are contained in these unique organelles. Romeo and Gennaro have demonstrated that the 10 large granules of bovine neutrophils contain potent microbicidal peptides that are structurally distinct from defensins (Gennaro, R., *et al.*, *Infect. Immun.* 57:3142-3146, 1989; Romeo, D., *et al.*, *J. Biol. Chem.* 263:9573-9575, 1988). These include three arginine-rich peptides, termed batenecins, which efficiently kill several gram positive and gram negative bacteria *in vitro*. Recently, the isolation and characterization of a 15 novel tridecapeptide amide, indolicidin, from bovine neutrophils was reported (Selsted, M.E., *et al.*, *J. Biol. Chem.* 267:4292-4295, 1992). This cationic peptide was shown to be unusually rich in tryptophan, and to have potent bactericidal activity against *E. coli* and *S. aureus*. More recently the isolation of 13  $\beta$ -defensins from bovine neutrophils demonstrated that these peptides are covalently dissimilar to defensins, while possessing 20 a similar folded conformation (Selsted, M.E., *et al.*, *J. Biol. Chem.* 268:6641-6648, 1993).

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**SUMMARY OF THE INVENTION**

The present invention provides peptides useful as antimicrobial agents. The invention arose from the discovery of a novel tridecapeptide from bovine peripheral blood granulocytes. The purified peptides and their carboxamide analogs have potent  
5 antibacterial, antiviral, antiprotozoal, and antifungal activities. These peptides, designated BGP-A and MGP-A, are effective compounds for use in human and/or veterinary medicine, or as agents in agricultural, food science, or industrial applications for example.

The details of the preferred embodiment of the present invention are set forth in the  
10 accompanying drawings and the description below. Once the details of the invention are known, numerous additional innovations and changes will become obvious to one skilled in the art.

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**BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 shows chromatographs of the purification of BGP-A. Figure 1a shows the gel filtration chromatography of bovine neutrophil granule extract. Figure 1b shows the reversed phase HPLC of the peak E fractions.

- 5 Figure 2 shows the analysis of purified BGP-A. Figure 2a shows the analytical RP-HPLC. Figure 2b shows the acid-urea gel of purified BGP-A.

Figure 3 shows the acid-urea PAGE of purified BGP-A and BGP-A-amide.

Figure 4 shows the cDNA nucleotide sequence (SEQ ID NO: 2) and the deduced precursor amino acid peptide sequence (SEQ ID NO: 3) of BGP-A.

- 10 Figure 5 shows the cDNA nucleotide sequence (SEQ ID NO: 4) and the deduced precursor amino acid peptide sequence (SEQ ID NO: 5) of MGP-A.

Figure 6 shows the mature BGP-A (SEQ ID NO: 6) and MGP-A (SEQ ID NO: 7) amino acid sequences. Hatched area indicates identical amino acids conserved between BGP-A and MGP-A. The consensus peptide amino acid sequence is identified as SEQ  
15 ID NO: 1.

Figure 7 shows the microbicidal activities of natural and synthetic BGP-A and synthetic BGP-A-amide.

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**DETAILED DESCRIPTION OF THE INVENTION**

Before the present nucleic and amino acid sequences, compositions, reagents and methods and uses thereof are described, it is to be understood that this invention is not limited to the particular compositions, reagents, sequences and methodologies described  
5 herein as such compositions, reagents, sequences and methodologies may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and that the terminology used herein is not intended to limit the scope of the present invention which will be limited only by the appended claims.

10 It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the," include plural referents unless the context clearly indicates otherwise. Thus, for example, reference to "a reagent" includes one or more of such different reagents, reference to "an antibody" includes one or more of such different antibodies,  
15 and reference to "the method" includes reference to equivalent steps and methods known to those of ordinary skill in the art that could be modified or substituted for the methods described herein.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention applies. Although any methods, compositions, reagents, sequences similar or  
20 equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods and materials are described herein. All publications mentioned herein are incorporated herein, including all figures, graphs, equations, illustrations, and drawings, to describe and disclose specific information for which the reference was cited in connection with.

25 The publications discussed above are provided solely for their disclosure before the filing date of the present application. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

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Throughout this description, the preferred embodiment and examples shown should be considered as exemplars, rather than as limitations on the present invention.

During the purification of bovine granulocyte  $\beta$ -defensins, antimicrobial activity associated with a small peptide was detected that was different from any previously characterized. Presented herein is the purification, sequencing, synthesis, cDNA isolation, and antimicrobial properties of BGP-A, a thirteen-residue peptide antibiotic expressed in bovine granulocytes. The cDNA for a mouse homolog of BGP-A, isolated from mouse bone marrow and designated MGP-A, is also presented. The deduced MGP-A precursor was remarkably similar to that of BGP-A. The present invention also teaches the synthesis and antimicrobial properties of BGP-A-amide and MGP-A-amide which are analogs of BGP-A and MGP-A respectively.

The invention provides peptide molecules, designated bovine granulocyte peptide -A (BGP-A) and mouse granulocyte peptide -A (MGP-A) and their synthetic carboxamides, designated BGP-A-amide and MGP-A-amide; which exhibit a broad range of antimicrobial and antiprotozoal activity and consequently, are effective antimicrobial agents. Polynucleotides encoding BGP-A and MGP-A represent a new class of antimicrobial peptide genes. As demonstrated by the high conservation of the precursor structure in a ruminant and a rodent, this gene family appears to be remarkably conserved. In a manner similar to the generating of indolicidin (Selsted, M.E., *et al.*, *Peptides: Chemistry and Biology*, ESCOM J.A. Smith and J.E. Rivier, 1992, pp. 905-907), the peptide is synthesized as a much larger prepropeptide and subsequently packaged in granules as the mature product of proteolytic processing. The methods used for the isolation and purification of BGP-A and MGP-A peptides are similar to those previously used for defensin-like peptides; such methods are taught in U.S. Patent Serial Nos. 4,453,252, 4,659,692, 4,705,777 and 5,242,902, all of which are incorporated by reference herein in their entirety.

As used herein, the term "antimicrobial activity" refers to the ability of a compound to inhibit or irreversibly prevent the growth of a microorganism. Such inhibition or

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prevention can be through a microbicidal action or microbistatic inhibition. Therefore, the term "microbicidal inhibition" or "inhibition of microbial growth" as used herein refers to the ability of the antimicrobial peptide to kill, or irrevocably damage the target organism. The term "microbistatic inhibition" as used herein refers to the growth of the target organism without death. Microbicidal or microbistatic inhibition can be applied to an environment either presently exhibiting microbial growth (i.e., therapeutic treatment) or an environment at risk of sustaining or supporting such growth (i.e., prevention or prophylaxis).

As used herein, the term "environment capable of sustaining or supporting microbial growth" refers to a fluid, tissue, space, organ, surface substance or organism where microbial growth can occur or where microbes can exist. Such environments can be, for example, animal tissue; skin or bodily fluids, water and other liquids, food, food products or food extracts, surfaces, crops and certain inanimate objects. It is not necessary that the environment promote the growth of the microbe, only that it permits its subsistence.

The antimicrobial, or antibacterial, activity of BGP-A or MGP-A can be measured against various pathogens by one of ordinary skill in the art. Microorganisms are grown to appropriate concentration, mixed with an appropriate medium, such as an agarose trypticase soy medium, and contacted with BGP-A or MGP-A. After appropriate incubation, the antimicrobial activity is apparent from clear zones surrounding the antibacterial samples. The clear zones are dependent upon the concentration of the peptide. Further methods of determination of antimicrobial activity are taught in Example 5 and in the section entitled "Materials and Methods" herein and are commonly known by those in the art.

Additionally, the minimum inhibitory concentrations (MIC) of BGP-A or MGP-A to effect antimicrobial activity can be determined for a number of different microorganisms according to standard techniques. Briefly, cells are grown overnight at about 37°C in appropriate bacterial media and diluted in the same medium to give

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concentrations of about  $10^4$  to  $10^5$  CFU/ml. The broth dilutions are set up in a 96 well microtiter plate, for example, mixing combinations of serially diluted microbes and peptides. After additions of serially diluted bacteria, or other microbes with serially diluted peptide concentrations, the plates are incubated overnight at about 37°C. The  
5 next day the plates are scored for the presence or absence of microbial growth in the wells, and the MIC is determined from the scoring.

As used herein, the term's BGP-A, BGP-A-amide, MGP-A and MGP-A-amide refer to peptides or peptidomimetics having generally about 8 to 20 amino acids which make up a chain having a net positive charge. In other words, these are cationic peptides. The  
10 peptides of the invention preferably have one or more aromatic amino acids. Illustrative peptide sequences are provided in Figs. 4-6 and as set forth in SEQ ID NOs: 1, 3, 5, 6 and 7.

The full length BGP-A cDNA is 688 nucleotides in length (SEQ ID NO: 2) with a predicted 21 kD precursor protein composed of 190 residues (SEQ ID NO: 3). Within  
15 the precursor peptide, 11 of the first 21 residues are hydrophobic and predict a signal peptide. The signal peptide domain is followed by an intervening propeptide region containing 156 residues. The final 13 residues of the precursor correspond to the mature BGP-A peptide sequence, YKIIQQWPHYRRV (SEQ ID NO: 6).

The full length MGP-A cDNA is 679 nucleotides in length (SEQ ID NO: 4) and  
20 predicts a precursor peptide (SEQ ID NO: 4) comprising signal pro-peptide domains similar to those described for BGP-A (Fig. 5). The mature peptide sequence predicted by the murine MGP-A cDNA is identical to BGP-A at 7 of 13 residues (YQVIQSWEHYRE) (Fig. 6; SEQ ID NO: 7). A consensus sequence between the mature BGP and MGP peptides is set forth in Fig. 6 where the hatched area indicates  
25 identical amino acids that are conserved between BGP-A and MGP-A and in SEQ ID NO: 1 having an amino acid sequence of YXXIXWXHYR, where X can be any amino acid. The peptides of the present invention include the SEQ ID NO: 1 consensus sequence. While not wanting to be bound by a particular theory, it is believed that the

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C-terminus should contain a net positive charge so that the molecule remains active. For example, SEQ ID NO: 1, 6 and 7 all end with an arginine (R) residue, SEQ ID NO: 6 ends with an arginine (R) and valine (V), and SEQ ID NO: 7 ends with a glutamic acid (E) residue. Given that the invention provides both the consensus sequence  
5 between mouse and bovine species and the individual DNA sequences encoding the peptides of the present invention, it would not require undue experimentation by the ordinary artisan to isolate homologous BGP/MGP sequences from other species, including human, porcine, ovine, etc., using the teachings supplied herein and methods common in the art (see Maniatis, *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold  
10 Spring Harbor Laboratory, Cold Spring Harbor, New York, current edition, incorporated herein by reference).

It should be appreciated that various modifications can be made to the BGP-A or MGP-A amino acid sequences without diminishing the antimicrobial activity of the peptides. It is intended that peptides or peptidomimetics of BGP-A or MGP-A exhibiting such  
15 modifications, including amino acid additions, deletions or substitutions are within the scope of the invention. As used herein, the term "substantially the same sequence" refers to a peptide sequence either identical to, or having considerable homology with, for example, the sequences BGP-A or MGP-A as shown in Figs. 4, 5, and 6 and in SEQ ID NOs: 1, 3, 5, 6 and 7. It is understood that limited modifications can be made to  
20 the peptide which result in enhanced function. Likewise, it is also understood that limited modifications can be made without destroying the biological function of the peptide and that only part of the entire primary structure may be required to affect activity. For example, minor modifications of these sequences that do not completely destroy the activity also fall within this definition and within the definition of the  
25 compound claimed as such. Modifications can include, for example, additions, deletions, or substitutions of amino acid residues, substitutions with compounds that mimic amino acid structure or function as well as the addition of chemical moieties such as amino and acetyl groups. The modifications can be deliberate or can be accidental such as through mutation in hosts that produce BGP-A or MGP-A peptides exhibiting

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antimicrobial activity. All these modifications are included as long as the peptide retains its antimicrobial activity.

In some cases, it may be desirable to incorporate one or more non-natural amino acids in the synthetic peptides of the present invention. Possible non-natural amino acids will usually have at least an N-terminus and a C-terminus and will have side chains that are either identical to or chemically modified or substituted from a natural amino acid counter part. An example of a non-natural amino acid is an optical isomer of a naturally-occurring L-amino acid. All peptides were synthesized using L amino acids, however, all D forms of the peptides can be synthetically produced. In addition, C-terminal derivatives can be produced, such as C-terminal methyl esters, to increase the antimicrobial activity of a peptide of the invention. Numerous modifications are contemplated according to this invention. Besides the obvious approach of replacement of specific residues in the natural sequence, an alternative embodiment involves synthesis of the peptide from D-amino acids thus reducing potential inactivation by proteases. Such means are well known in the art. (See, for example, Wade *et al.*, *PNAS, USA* 87:4761-4765, 1990.) Examples of chemical modification or substitutions may include hydroxylation or fluorination of C-H bonds within natural amino acids. Such techniques are used in the manufacture of drug analogs of biological compounds and are known to those of ordinary skill in the art. In a preferred embodiment the modification of the peptides of the invention comprises modification by a carboxy terminal amide. Those of skill in the art can make similar substitutions to achieve peptides with greater antimicrobial activity and a broader host range. For example, the invention includes the peptides as set forth in SEQ ID NO:1, 3, 5, 6 and 7, as well as analogues, derivatives or functional fragments thereof, as long as the antimicrobial activity of the peptide remains. Minor modifications of the primary amino acid sequence of the peptides of the invention may result in peptides which have substantially equivalent antimicrobial activity as compared to the specific peptides as set forth in the SEQ ID NOs: 1, 3, 5, 6 and 7 described herein. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the peptides produced by these modifications are included herein as long as the antimicrobial biological

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activity of the original peptide still exists. BGP-A or MGP-A peptides of the present invention also include functional fragments of the peptide or functional fragments of the nucleic acid sequence encoding the peptide, as long as the activity of BGP-A or MGP-A remains. Smaller peptides containing the biological activity of BGP-A or MGP-A are also included in the invention as are smaller nucleic acid sequences encoding for all or a functional fragment of the peptide. The relative effectiveness of the functional fragments of the peptide or nucleic acid sequences encoding for functional fragments of the peptides of the invention can be readily determined by one of skill in the art by establishing the sensitivity of a microorganism to the peptide fragment. The effectiveness of the peptide functional fragments is assessed by measuring the potential microbicidal or microbistatic activity of the fragment or nucleic acid sequence encoding such a fragment as measured relative to the microbicidal ability of the BGP-A or MGP-A peptides of SEQ ID NO: 6 or 7 respectively. Testing is carried out as described in the section titled "Antimicrobial Assay" in the Materials and Methods section herein and in Example 5 of the present invention or by other standard antimicrobial tests (e.g., MIC) commonly known to those in the art.

Further, deletion of one or more amino acids can also result in a modification of the structure of the resultant peptide without significantly altering its biological activity. This can lead to the development of a smaller active peptide which would also have utility. For example, amino or carboxy terminal amino acids which may not be required for biological activity of the particular peptide can be removed. Peptides of the invention include any analog, homolog, mutant, isomer or derivative of the peptides disclosed in the present invention, so long as the bioactivity as described herein is remains. The methods and compositions of the present invention may also employ synthetic non-peptide compositions that have biological activity functionally comparable to that of BGP-A, MGP-A, BGP-A-Amide, or MGP-A-Amide. By "functionally comparable," it is meant that the shape, size, flexibility, and electronic configuration of the non-peptide molecule are such that the biological activity of the molecule is similar to the BGP-A, MGP-A, BGP-A-Amide, or MGP-A-Amide peptides. In particular, the non-peptide molecules should display comparable antimicrobial activity. Such non-

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peptide molecules can be small molecules having a molecular weight in the range of about 100 to 1000 Daltons. The use of such small molecules is advantageous in the preparation of pharmacological compositions.

The identification of such non-peptide analog molecules can be performed using techniques known in the art of drug design. Such techniques include, but are not limited to, self-consistent field (SCF) analysis, configuration interaction (CI) analysis, and normal mode dynamics computer analysis, all of which are well described in the scientific literature. See, e.g., Rein et al., *Computer-Assisted Modeling of Receptor-Ligand Interactions*, Alan Liss, N.Y., (1989). Preparation of the identified compounds will depend on the desired characteristics of the compounds will involve standard chemical synthetic techniques. See, Cary et al., *Advanced Organic Chemistry*, part B, Plenum Press, New York (1983).

The term "conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine, and the like. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted peptide also immunoreact with the unsubstituted peptide.

The BGP-A or MGP-A peptides of the present invention can be synthesized by methods well known in the art, such as through the use of automatic peptide synthesizers, by recombinant methods or well-known manual methods of peptide synthesis. In addition, they can be purified from natural sources such as white blood cells and from bone marrow of a vertebrate, preferably of mammalian origin. Such cells or tissues can be obtained by means well known to those skilled in the art.

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The term "substantially pure" as used herein refers to BGP-A or MGP-A nucleic acid or protein which is substantially free of other proteins, lipids, carbohydrates or other materials with which it is naturally associated or that the peptide or protein so designated has been separated from its *in vivo* cellular environment. Because of the separation and purification, the substantially pure peptides and proteins are useful in ways that the non-separated impure peptides or proteins are not. One skilled in the art can purify BGP-A or MGP-A using standard techniques for protein purification. The substantially pure peptide will yield a single major band on an acid-urea gel. The purity of the BGP-A or MGP-A peptide can also be determined by amino-terminal amino acid sequence analysis and analytical RP-HPLC.

The invention also provides polynucleotides encoding the BGP-A or MGP-A protein. These polynucleotides include DNA, cDNA and RNA sequences which encode BGP-A or MGP-A. It is understood that all polynucleotides encoding all or a portion of BGP-A or MGP-A are also included herein, as long as they encode a peptide with BGP-A or MGP-A activity. Such polynucleotides include naturally occurring, synthetic, and intentionally manipulated polynucleotides. For example, BGP-A or MGP-A polynucleotide may be subjected to site-directed mutagenesis. The polynucleotides of the invention include sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the invention as long as the amino acid sequence of BGP-A or MGP-A peptide encoded by the nucleotide sequence is functionally unchanged. The polynucleotide encoding BGP-A or MGP-A includes the nucleotide sequence in FIGURE 4 and 5 (SEQ ID NOs: 2 and 4), as well as complementary nucleic acid sequences. A complementary sequence may include an antisense nucleotide. When the sequence is RNA, the deoxynucleotides A, G, C, and T of SEQ ID Nos: 2 and 4 are replaced by ribonucleotides A, G, C, and U, respectively. Also included in the invention are fragments of the above-described nucleic acid sequences that are at least 15 bases in length, which is sufficient to permit the fragment to selectively hybridize to DNA (SEQ ID NOs: 2 and 4) that encodes the protein of FIGURE 4 and 5 (SEQ ID NOs: 3 and 5), under physiological conditions.

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Also, provided by this invention are the nucleic acid sequences encoding the BGP-A or MGP-A peptides, vectors and host cells containing them and methods of expression to provide recombinantly produced peptides. This method comprises growing the host cell containing a nucleic acid encoding a peptide under suitable conditions such that the  
5 nucleic acid is transmitted and/or translated and isolating the peptide so produced.

After the peptide of this invention is isolated, nucleic acids encoding the peptides are isolated by methods well known in the art, *infra*. These isolated nucleic acids can be ligated into vectors and introduced into suitable host cells for expression. Methods of ligation and expression of nucleic acids within cells are well known in the art, (see  
10 Maniatis, *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, current edition, incorporated herein by reference).

Specifically disclosed herein is a cDNA sequence containing the active portion of the BGP-A or MGP-A coding sequence. One of skill in the art could now use this sequence  
15 to isolate other full length clones. The full length BGP-A cDNA is 688 nucleotides in length (SEQ ID NO: 2) and predicts a 21 kD precursor composed of 190 residues (Fig. 4; SEQ ID NO: 3). Within the BGP-A precursor, 11 of the first 21 residues are hydrophobic and predict a signal peptide (Von Heijne, G., *Eur. J. Biochem.* 133:17-21, 1983). The signal peptide domain is followed by an intervening propeptide region  
20 containing 156 residues. The final 13 residues of the precursor correspond to the mature BGP-A peptide sequence (SEQ ID NO: 6). The full-length MGP-A cDNA is 679 nucleotides in length (SEQ ID NO: 4) and predicts a precursor comprising signal pro-peptide domains similar to those described for BGP-A (Fig. 5; SEQ ID NO: 5). Based on this similarity, this sequence isolated from murine bone marrow cDNA is designated  
25 as murine neutrophil peptide A (MGP-A; Fig. 5; SEQ ID NOs: 5 and 7). The mature peptide sequence predicted by the murine cDNA is identical to BGP-A at 7 of 13 residues (Fig. 6; SEQ ID NO: 7). The hatched area in Figure 6 indicates identical amino acids conserved between BGP-A and MGP-A. The consensus peptide amino acid sequence is YXXIQXWXHYR (SEQ ID NO: 1), where X can be any amino acid.

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DNA sequences of the invention can be obtained by several methods. For example, the DNA can be isolated using hybridization techniques which are well known in the art. These include, but are not limited to: 1) hybridization of genomic or cDNA libraries with probes to detect homologous nucleotide sequences, 2) polymerase chain reaction

5 (PCR) on genomic DNA or cDNA using primers capable of annealing to the DNA sequence of interest, and 3) antibody screening of expression libraries to detect cloned DNA fragments with shared structural features. The sequences of a pair of nucleic acid molecules (or two regions within a single nucleic acid molecule) are said to be "complementary" to each other if base pairing interactions can occur between each

10 nucleotide of one of the members of the pair and each nucleotide of the other member of the pair. A pair of nucleic acid molecules (or two regions within a single nucleic acid molecule) are said to "hybridize" to each other if they form a duplex by base pairing interactions between them. As known in the art, hybridization between nucleic acid pairs does not require complete complementarity between the hybridizing regions,

15 but only that there is a sufficient level of base pairing to maintain the duplex under the hybridization conditions used.

Hybridization reactions are typically carried out under low to moderate stringency conditions, in which specific and some nonspecific interactions can occur. After hybridization, washing can be carried out under moderate or high stringency conditions

20 to eliminate nonspecific binding. As known in the art, optimal washing conditions can be determined empirically, *e.g.*, by gradually increasing the stringency. Condition parameters that can be changed to affect stringency include, *e.g.*, temperature and salt concentration. In general, the lower the salt concentration and the higher the temperature, the higher the stringency. For example, washing can be initiated at a low

25 temperature (*e.g.*, room temperature) using a solution containing an equivalent or lower salt concentration as the hybridization solution. Subsequent washing can be carried out using progressively warmer solutions having the same salt solution. Alternatively, the salt concentration can be lowered and the temperature maintained in the washing step, or the salt concentration can be lowered and the temperature increased. Additional

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parameters can be altered to affect stringency, including, *e.g.*, the use of a destabilizing agent, such as formamide.

In nucleic acid hybridization reactions, the conditions used to achieve a particular level of stringency will vary, depending on the nature of the nucleic acids being hybridized.

- 5 For example, the length, degree of complementarity, nucleotide sequence composition (*e.g.*, GC v. AT content), and nucleic acid type (*e.g.*, RNA v. DNA) of the hybridizing regions of the nucleic acids can be considered in selecting hybridization conditions. An additional consideration is whether one of the nucleic acids is immobilized, for example, on a filter.
- 10 An example of progressively higher stringency conditions is as follows: 2 x SSC/0.1% SDS at about room temperature (hybridization conditions); 0.2 x SSC/0.1% SDS at about room temperature (low stringency conditions); 0.2 x SSC/0.1% SDS at about 42°C (moderate stringency conditions); and 0.1 x SSC at about 68°C (high stringency conditions). Washing can be carried out using only one of these conditions, *e.g.*, high
- 15 stringency conditions, or each of the conditions can be used, *e.g.*, for 10-15 minutes each, in the order listed above, repeating any or all of the steps listed. However, as mentioned above, optimal conditions will vary, depending on the particular hybridization reaction involved, and can be determined empirically.

- Preferably the BGP-A or MGP-A polynucleotide of the invention is derived from a
- 20 mammalian organism, and most preferably from a mouse, cow, or human. Screening procedures which rely on nucleic acid hybridization make it possible to isolate any gene sequence from any organism, provided the appropriate probe is available. Oligonucleotide probes, which correspond to a part of the sequence encoding the protein in question, can be synthesized chemically. This requires that short, oligopeptide
- 25 stretches of amino acid sequence must be known. The DNA sequence encoding the protein can be deduced from the genetic code, however, the degeneracy of the code must be taken into account. It is possible to perform a mixed addition reaction when the sequence is degenerate. This includes a heterogeneous mixture of denatured double-

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stranded DNA. For such screening, hybridization is preferably performed on either single-stranded DNA or denatured double-stranded DNA. Hybridization is particularly useful in the detection of cDNA clones derived from sources where an extremely low amount of mRNA sequence relating to the peptide of interest is present. In other words, by using stringent hybridization conditions directed to avoid nonspecific binding, it is possible, for example, to allow the autoradiographic visualization of a specific cDNA clone by the hybridization of the target DNA to that single probe in the mixture which is its complete complement (Wallace, *et al.*, *Nucl. Acid Res.*, 9:879, 1981).

Therefore, given a partial DNA sequence of the BGP-A or MGP-A gene of interest, one of skill in the art would be able to prepare probes for isolation of a full length cDNA clone, without undue experimentation (see for example, Ausubel, *et al.*, *Current Protocols in Molecular Biology*, Units 6.3-6.4, Greene Publ., 1994; Maniatis, *et al.*, *Molecular Cloning*, Cold Spring Harbor Laboratories, current edition).

The complement of specific DNA sequences encoding BGP-A or MGP-A can also be obtained by: 1) isolation of double-stranded DNA sequences from the genomic DNA; 2) chemical manufacture of a DNA sequence to provide the necessary codons for the peptide of interest; and 3) *in vitro* synthesis of a double-stranded DNA sequence by reverse transcription of mRNA isolated from a eukaryotic donor cell. In the latter case, a double-stranded DNA complement of mRNA is eventually formed which is generally referred to as cDNA. Of the three above-noted methods for developing specific DNA sequences for use in recombinant procedures, the isolation of genomic DNA isolates is the least common. This is especially true when it is desirable to obtain the microbial expression of mammalian peptides due to the presence of introns.

The synthesis of DNA sequences is frequently the method of choice when the entire sequence of amino acid residues of the desired peptide product is known. When the entire sequence of amino acid residues of the desired peptide is not known, the direct synthesis of DNA sequences is not possible and the method of choice is the synthesis of cDNA sequences. Among the standard procedures for isolating cDNA sequences of

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- interest is the formation of plasmid- or phage-carrying cDNA libraries which are derived from reverse transcription of mRNA which is abundant in donor cells that have a high level of genetic expression. When used in combination with polymerase chain reaction technology, even rare expression products can be cloned. In those cases where
- 5 significant portions of the amino acid sequence of the peptide are known, the production of labeled single or double-stranded DNA or RNA probe sequences duplicating a sequence putatively present in the target cDNA may be employed in DNA/DNA hybridization procedures which are carried out on cloned copies of the cDNA which have been denatured into a single-stranded form (Jay, *et al.*, *Nucl. Acid*, 11:2325, 1983).
- 10 Several types of vectors are available and can be used to practice this invention, e.g., plasmid, DNA and RNA viral vectors, baculoviral vectors, and vectors for use in yeast. When the vector is a plasmid, it generally contains a variety of components including promoters, signal sequences, phenotypic selection genes, origins of replication sites, and other necessary components as are known to those of skill in the art.
- 15 Promoters most commonly used in prokaryotic vectors include the lac Z promoter system, the alkaline phosphatase pho A promoter, the bacteriophage  $\lambda$ PL promoter (a temperature sensitive promoter), the tac promoter (a hybrid trp-lac promoter regulated by the lag repressor), the tryptophan promoter, and the bacteriophage T7 promoter.
- One other useful component of vectors used to practice this invention is a signal
- 20 sequence. This sequence is typically found immediately 5' to the nucleic acid encoding the peptide, and will thus be transcribed at the amino terminus of the fusion protein. However, in certain cases, the signal sequence has been demonstrated to be at positions other than 5' to the gene encoding the protein to be secreted. This sequence targets the protein to which it is attached across the inner membrane of the bacterial cell. The
- 25 DNA encoding the signal sequence can be obtained as a restriction endonuclease fragment from any nucleic acid encoding a peptide that has a signal sequence. Suitable prokaryotic signal sequences can be obtained from genes encoding, for example Lamb or OmpF (Wong, *et al.*, *Gene* 68:193, 1983), MalE, PhoA, OmpA and other genes. A

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preferred prokaryotic signal sequence for practicing this invention is the *E. coli* heat-stable enterotoxin II (STII) signal sequence as described by Chang, *et al*, *Gene* 55:189, 1987.

Another useful component of the vectors used to practice this invention is a phenotypic selection gene. Typical phenotypic selection genes are those encoding proteins that confer antibiotic resistance upon the host cell. By way of illustration, the ampicillin resistance gene (amp) and the tetracycline resistance gene (tet) are readily employed for this purpose.

Construction of suitable vectors comprising the aforementioned components as well as the gene encoding the desired peptide are prepared using standard recombinant DNA procedures. Isolated DNA fragments to be combined to form the vector are cleaved, tailored, and ligated together in a specific order and orientation to generate the desired vector.

The DNA is prepared according to standard procedures (see Maniatis, *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, current edition, incorporated herein by reference). If the DNA fragment is to be ligated into a vector, the vector is at first linearized by cutting with the appropriate restriction endonucleases. The linearized vector can then be treated with alkaline phosphatase or calf intestinal phosphatase. The phosphatasing prevents self-ligation of the vector during the ligation step.

After ligation, the vector with the heterologous gene now inserted is transformed into a suitable host cell. Suitable prokaryotic host cells include *E. coli* strain JM101, *E. coli* K12 strain 294 (ATCC number 31,446), *E. coli* strain W3110 (ATCC number 27,325), *E. coli* X1776 (ATCC number 31, 537), *E. coli* XL-1Blue (Stratagene), and *E. coli* B; however, many other strains of *E. coli*, such as HB101, NM522, NM538, NM539 and many other species and genera of prokaryotes can be used as well. Besides the *E. coli* strains listed above, bacilli such as *Bacillus subtilis*, other enterobacteriaceae such as

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*Salmonella typhimurium* or *Serratia marcesans* and various *Pseudomonas* species can all be used as hosts.

Transformation of prokaryotic cells is readily accomplished using calcium chloride or other methods well known to those skilled in the art. Electroporation (Neumann, *et al.*, 5 *EMBO J.* 1:841, 1982) also can be used to transform these cells. The transformed cells are selected by growth on an antibiotic, commonly tetracycline (tet) or ampicillin (amp), to which they are rendered resistant due to the presence of tet and/or amp resistance genes on the vector.

After selection of the transformed cells, these cells are grown in culture and the plasmid 10 DNA (or other vector with the foreign gene inserted) is then isolated. Plasmid DNA can be isolated using methods known in the art. This purified plasmid DNA is then analyzed by restriction mapping and/or DNA sequencing.

Following procedures outlined above, mammalian cell lines such as myeloma (P3-653), hybridoma (SP2/0), Chinese Hamster Ovary (CHO), Green monkey kidney (COSI) and 15 murine fibroblasts (L492) are suitable host cells for peptide expression. These "mammalian" vectors can include a promoter, an enhancer, a polyadenylation signal, signal sequences and genes encoding selectable markers such as geneticin (neomycin resistance), mycophenolic acid (xanthine guanine phosphoribosyl transferase) or histidinol (histidinol dehydrogenase).

20 Suitable promoters for use in mammalian host cells include, but are not limited to, Ig Kappa, Ig Gamma, Cytomegalovirus (CMV) immediate early, Rous Sarcoma Virus (RSV), Simian virus 40 (SV40) early, mouse mammary tumor (MMTV) virus and metallothionein. Suitable enhancers include, but are not limited to, Ig Kappa, Ig Heavy, CMV early and SV40. Suitable polyadenylation sequences include Ig Kappa, Ig Gamma 25 or SV40 large T antigen. Suitable signal sequences include Ig Kappa, Ig Heavy and human growth hormone (HGH).

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When the vector is baculovirus, suitable promoters and enhancer sequences include, but are not limited to, AcMGPV polyhedrin, AcMGPV ETL and AcMGPV p10 sequences. One particularly suitable polyadenylation signal is the polyhedrin AcMGPV. Ig Kappa, Ig Heavy and AcMGPV are examples of suitable signal sequences. These vectors are  
5 useful in the following insect cell lines, among others: SF9, SF21 and High 5.

Alternatively, the peptides can be expressed in yeast strains such as PS23-6A, W301-18A, LL20, D234-3, INVSC1, INVSC2, YJJ337. Promoter and enhancer sequences such as gal 1 and pEFT-1 are useful. Vra-4 also provides a suitable enhancer sequence. Sequences useful as functional "origins of replication" include ars1 and 2 $\mu$  circular  
10 plasmid.

The invention includes antibodies that are immunoreactive with BGP-A or MGP-A peptides or fragments thereof. Antibodies which consist essentially of pooled monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibody preparations are provided. Monoclonal antibodies are made from antigen  
15 containing fragments of the protein by methods well known to those skilled in the art (Kohler, *et al.*, *Nature*, 256:495, 1975). Anti-BGP-A or MGP-A antibodies can be made by methods conventional in the art. For example, polyclonal antiserum can be raised in appropriate animals, such as rabbits, mice, or rats. BGP-A or MGP-A peptides, either synthetically obtained or naturally obtained, can be used to immunize the animal. The  
20 immunogen can then be used to immunize animals by means well known to those skilled in the art. Serum samples are collected until the anti-BGP-A or MGP-A titer is appropriate. Various fractions of the antisera, such as IgG, can be isolated by means well known in the art. Alternatively, BGP-A or MGP-A immunogens can be used to obtain monoclonal antibodies, again by means well known in the art. (See, for example,  
25 Harlow *et al.*, *Antibodies: A Laboratory Manual*, Cold Springs Harbor Laboratory, 1988.)

Anti-BGP-A or MGP-A antibodies can be used to detect the presence of BGP-A or MGP-A in biological samples, such as histological samples. An appropriate detectable

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second antibody can be used to identify the primary antibody attached to the BGP-A or MGP-A by visualization. Means of detection include the use of radioactive nucleotides or enzyme substrates such as peroxidase. For example, anti-BGP-A was produced by standard methods and shown to stain bone marrow preparations from cattle (cytological  
5 sample). In particular, granulocytes (e.g., eosinophils) were stained heavily for BNP-A.

The term "antibody" as used in this invention includes intact molecules as well as fragments thereof, such as Fab, Fab', F(ab')<sub>2</sub>, and Fv that can bind the epitopic determinant. These antibody fragments retain some ability selectively to bind with its antigen or receptor and are defined as follows:

- 10 (1) Fab, the fragment that contains a monovalent antigen-binding fragment of an antibody molecule can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and part of one heavy chain;
- (2) Fab', the fragment of an antibody molecule can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and  
15 part of the heavy chain; two Fab' fragments are obtained per antibody molecule;
- (3) (Fab')<sub>2</sub>, the fragment of the antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; F(ab')<sub>2</sub> is a dimer of two Fab' fragments held together by two disulfide bonds;
- (4) Fv, defined as a genetically engineered fragment containing the variable region  
20 of the light chain and the variable region of the heavy chain expressed as two chains; and

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(5) Single chain antibody ("SCA"), defined as a genetically engineered molecule containing the variable region of the light chain, the variable region of the heavy chain, linked by a suitable peptide linker as a genetically fused single chain molecule.

- 5 Methods of making these fragments are known in the art. (See for example, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York (current edition), incorporated herein by reference).

As used in this invention, the term "epitope" means any antigenic determinant on an antigen to which the paratope of an antibody binds. Epitopic determinants usually  
10 consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics.

If needed, polyclonal or monoclonal antibodies can be further purified, for example, by binding to and elution from a matrix to which the peptide or a peptide to which the  
15 antibodies are raised is bound. Those of skill in the art will know of various techniques common in the immunology arts for purification and/or concentration of polyclonal antibodies, as well as monoclonal antibodies (See, e.g., Coligan, *et al.*, Unit 9, *Current Protocols in Immunology*, Wiley Interscience, current edition, incorporated by reference).

- 20 It is also possible to use the anti-idiotypic technology to produce monoclonal antibodies that mimic an epitope. For example, an anti-idiotypic monoclonal antibody made to a first monoclonal antibody will have a binding domain in the hypervariable region that is the "image" of the epitope bound by the first monoclonal antibody.

The phrase "purified antibody" means an antibody that is at least 60%, by weight, free  
25 from proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably 90%, and most

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preferably at least 99%, by weight, an antibody, *e.g.*, an anti-BGP-A specific antibody. A purified antibody may be obtained, for example, by affinity chromatography using recombinantly-produced protein or conserved motif peptides and standard techniques. The invention can employ not only intact monoclonal or polyclonal antibodies, but also  
5 an immunologically-active antibody fragment, such as a Fab, Fab' or (Fab')<sub>2</sub> fragments, or a genetically engineered Fv fragment (Ladner *et al.*, U.S. Patent No. 4,946,788).

"Specifically binds" means an antibody that recognizes and binds a specified protein, *e.g.*, an anti-BGP-A, specific antibody or anti-MGP-A specific antibody, which does not substantially recognize and bind other molecules in a sample which naturally includes  
10 protein.

It should be understood that the compositions of the present invention have activity against many microorganisms, such as fungi, bacteria (both gram positive and negative), and protozoa and viruses. Different compositions will have differing degrees of activities toward different organisms. The peptides of the present invention may also  
15 be combined with other proteins to act as preservatives to protect the proteins against bacterial degradation. Alternatively, the subject peptides or compositions may be used as preservatives and disinfectants in many formulations, such as contact lens solutions, ointments, shampoos, medicaments, foods, and the like. The amount of peptide employed in the compositions may vary depending upon the nature of the other  
20 components, how much protection is required and the intended use of the composition.

In a preferred embodiment, the present invention provides administration of a therapeutic amount of an antimicrobial peptide of the invention. One or more of the peptides disclosed herein, may have utility as antifungal agents, either alone, or as lipid fascicle preparations. The latter approach has been used with success with the non-  
25 peptide antifungal drug amphotericin. Specific applications would be dependent on the pathogen targeted. For example, *C. albicans*, the common cause of mucocutaneous fungal disease in AIDS patients, which is extremely susceptible to several  $\beta$ -defensins, might be controlled in these individuals more effectively by a BGP-A or MGP-A based

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therapeutic or in combination with existing first line drugs. Similarly, BGP-A or MGP-A may be used therapeutically in veterinary medicine. One advantage of the therapeutic use of the present invention is that the peptides exhibit low immunogenicity.

5 BGP-A or MGP-A, either purified from natural sources or synthetic, can be administered to a subject in need of therapy by various means, including oral administration, preferably in a slow-release type formulation that will avoid release within the stomach. Alternatively, they can be administered through a nasal gastric incubation or transabdominal catheter. Individual species of BGP-A or MGP-A can be administered singly or a combination can be administered simultaneously or sequentially  
10 and also with other antimicrobial compositions.

The invention further provides a pharmaceutical composition for treating a human bacterial or fungal infection that comprises the purified peptide of the invention in an amount effective to treat a human bacterial or fungal infection and a pharmaceutically acceptable carrier.

15 The method of inhibiting the growth of bacteria may further include the addition of antibiotics for combination or synergistic therapy. The appropriate antibiotic administered will typically depend on the susceptibility of the bacteria such as whether the bacteria is gram negative or gram positive, and will be easily discernable by one of skill in the art. Examples of particular classes of antibiotics useful for synergistic  
20 therapy with the peptides of the invention include aminoglycosides (*e.g.*, tobramycin), penicillins (*e.g.*, piperacillin), cephalosporins (*e.g.*, ceftazidime), fluoroquinolones (*e.g.*, ciprofloxacin), carbapenems (*e.g.*, imipenem), tetracyclines and macrolides (*e.g.*, erythromycin and clarithromycin). The method of inhibiting the growth of bacteria may further include the addition of antibiotics for combination or synergistic therapy. The  
25 appropriate antibiotic administered will typically depend on the susceptibility of the bacteria such as whether the bacteria is gram negative or gram positive, and will be easily discernable by one of skill in the art. Further to the antibiotics listed above, typical antibiotics include aminoglycosides (amikacin, gentamicin, kanamycin,

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netilmicin, tobramycin, streptomycin, azithromycin, clarithromycin, erythromycin, erythromycin estolate/ethylsuccinate/glucetate/lactobionate/stearate), beta-lactams such as penicillins (e.g., penicillin G, penicillin V, methicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin, ampicillin, amoxicillin, ticarcillin, carbenicillin, mezlocillin, azlocillin and  
5 piperacillin), or cephalosporins (e.g., cephalothin, cefazolin, cefaclor, cefamandole, cefoxitin, cefuroxime, cefonicid, cefmetazole, cefotetan, cefprozil, loracarbef, cefetamet, cefoperazone, cefotaxime, ceftizoxime, ceftriaxone, ceftazidime, cefepime, cefixime, cefpodoxime, and cefsulodin). Other classes of antibiotics include carbapenems (e.g., imipenem), monobactams (e.g., aztreonam), quinolones (e.g., fleroxacin, nalidixic acid,  
10 norfloxacin, ciprofloxacin, ofloxacin, enoxacin, lomefloxacin and cinoxacin), tetracyclines (e.g., doxycycline, minocycline, tetracycline), and glycopeptides (e.g., vancomycin, teicoplanin), for example. Other antibiotics include chloramphenicol, clindamycin, trimethoprim, sulfamethoxazole, nitrofurantoin, rifampin and mupirocin.

In certain embodiments of the invention, the treatment of the soluble proteins comprises  
15 size exclusion chromatography, ion-exchange chromatography, or reverse phase, high performance, liquid chromatography. It will be appreciated by one skilled in the art, however, that treatment of soluble proteins to purify peptides may be accomplished by many methods known to those skilled in the art, all of which are contemplated by this invention. Further, in one embodiment of the invention, the treatment of granulocytes  
20 to recover granules comprises density gradient centrifugation.

The invention also provides a composition that comprises the purified peptide in an amount effective to kill bacteria or fungi and a suitable carrier. Such composition may be used in numerous ways to combat bacteria or fungi, for example, in household or laboratory antimicrobial formulations using carriers well known in the art.

25 The compositions of the present invention can comprise the BGP-A, BGP-A-Amide, MGP-A, or MGP-A-Amide, either singly or in combination, incorporated in a physiologically-acceptable-carrier suitable for topical application. The compositions may contain from about 10 ug/ml to 2000 ug/ml, preferably 50 ug/ml to 500 ug/ml. The

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nature of the carrier will vary depending on the intended area of application. For application to the skin, a cream or an ointment base is usually preferred with suitable bases including lanolin, Silvadene™ (Marion; particularly for the treatment of burns) Aquaphor™ (Duke Laboratories, South Norwalk, Conn.), and the like. It will also be possible to incorporate the BGP-A, BGP-A-Amide, MGP-A, or MGP-A-Amide peptides in natural and synthetic bandages and other wound dressings to provide for continuous exposure of a wound to the peptides. Aerosol applicators may also find use with the present invention.

Where the peptides are to be used as antimicrobial agents, they can be formulated in buffered aqueous media containing a variety of salts and buffers. The salts will for the most parts be alkali and alkaline earth halides, phosphates and sulfates, e.g., sodium chloride, potassium chloride or sodium sulfate. Various buffers may be used, such as citrate, phosphate, HEPES, Tris or the like to the extent that such buffers are physiologically acceptable to the host that is being treated.

Various excipients or other additives may be used, where the compounds are formulated as lyophilized powders, for subsequent use in solution. The excipients may include various polyols, inert powders or other extenders.

Depending on the nature of the formulation and the host, the subject compounds may be administered in a variety of ways. The formulations may be applied topically, by injection, e.g., intravenously, intraperitoneal, nasopharyngeal, etc.

In another aspect of the invention, compositions comprising the purified peptide of the invention in a microbicidal effective amount and a suitable carrier or pharmaceutical composition, or pharmaceutically acceptable carrier may additionally comprise a detergent. The addition of a detergent to such peptide compositions is useful to enhance the antibacterial, antiviral, or antifungal characteristics of the novel peptide of the invention. Although any suitable detergent may be used, the presently preferred detergent is a nonionic detergent, such as Tween 20 or 1% NP40.

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The invention also provides a pharmaceutical formulation or composition for treating a human microbial, bacterial, viral, or fungal infection that comprises the purified peptide of the invention or a gene delivery and gene expression vector that can deliver an effective amount of peptide in an amount effective to treat a human microbial  
5 bacterial, viral, or fungal infection incorporated into a pharmaceutically acceptable liposome or other delivery vehicle.

"Formulation" means a composition capable of gene delivery and gene expression, which can deliver a nucleotide sequence to, or directly into, a target cell whereupon the formulation containing the nucleotide sequence is incorporated on the cytoplasmic side  
10 of the outermost membrane of the target cell and capable of achieving gene expression so that detectable levels of gene expression of the delivered nucleotide sequence are expressed in the target cell. More preferably, after delivery into the cytoplasmic side of the cell membrane the composition is subsequently transported, without undergoing endosomal or lytic degradation, into the nucleus of the target cell in a functional state  
15 capable of achieving gene expression so that detectable levels of gene expression of the delivered nucleotide sequence are expressed in the target cell. Expression levels of the gene or nucleotide sequence inside the target cell can provide gene expression for a duration of time and in an amount such that the nucleotide product therein can provide a biologically beneficially effective amount of a gene product or in such an amount as  
20 to provide a functionally beneficial biological effect. As used herein, the term formulation can refer to, but is not limited by (either explicitly or implicitly) the following examples: (1) liposome or liposome reagents or liposomal compositions either cationic, anionic or neutral in net character and net charge; (2) DNA, nucleic acid or a nucleic acid expression vector ionically complexed with a polycation/s and a ligand/s  
25 such that after attachment of the [DNA + Polycation + Ligand] composition to a cell surface receptor on a target cell via the ligand, the [DNA + Polycation + Ligand] composition can be endocytosed into the target cell and the DNA is subsequently decoupled from the ligand and polycation and delivered to the cell nucleus in a functional condition for subsequent expression. Various alterations in the composition  
30 can be envisioned by those of ordinary skill in the art such as including peptide

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sequences that (a) protect the composition from endosomal lysis after incorporation into the target cell by allowing the composition to leave the lysosomal vesicle, or (b) which act as a nuclear targeting agent, chaperoning the nucleic acid through the pores of the nuclear envelope and into the nucleus of the cell. Similar reagents, which have been previously described, are the asialoglycoprotein-polylysine conjugations (Wu *et al.*, *J. Biol. Chem.* 263:14621, 1988; Wu *et al.*, *J. Biol. Chem.* 264:16985, 1989); (3) naked nucleic acid; (4) compacted nucleic acid or a compacted reagent; or (5) plasmid or naked DNA that can be microinjected (Wolff *et al.*, *Science* 247:1465, 1990); (6) nucleic acid in a viral or retroviral vector composition; and (7) colloidal dispersions (Felgner *et al.*, *Proc. Natl. Acad. Sci. USA* 84:7413, 1987; Ono *et al.*, *Neuroscience Lett.* 117:259, 1990; Brigham *et al.*, *Am. J. Med. Sci.* 298:278, 1989; Staubinger and Papahadjopoulos, *Meth. Enz.* 101:512, 1983). One of ordinary skill in the art will recognize that other compositions for the delivery of nucleotide sequences to target cells may be envisioned.

It will be readily understood by those skilled in the art that any suitable pharmaceutically acceptable liposome may be used as a vehicle for the peptide of the present invention. Such liposomal compositions have activity against many microorganisms similar to the activity of other compositions of this invention discussed in more detail above. Additionally, these compositions may be administered in a variety of conventional and well-known ways as is also discussed in greater detail above.

"Therapeutically effective" as used herein, refers to an amount of formulation, composition, or reagent in a pharmaceutical acceptable carrier that is of sufficient quantity to ameliorate the state of the patient or animal so treated. "Ameliorate" refers to a lessening of the detrimental effect of the disease state or disorder in the recipient of the therapy. The subject of the invention is preferably a human, however, it can be envisioned that any animal can be treated in the method of the instant invention. The term "modulate" means enhance, inhibit, alter, or modify the expression or function of antimicrobial activity in combination with a pharmaceutically acceptable carrier.

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Pharmaceutically acceptable carrier preparations for administration include sterile or aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. The active therapeutic ingredient is often mixed with excipients that are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients include water, saline, dextrose, glycerol and ethanol, or combinations thereof. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present such as, for example, antimicrobial, antioxidants, chelating agents, and inert gases and the like.

Another therapeutic approach included within the invention involves direct administration of reagents or compositions by any conventional administration techniques (for example but not restricted to local injection, inhalation, or administered systemically), to the subject with a microbial, bacterial, viral or fungal disorder. The reagent, formulation or composition may also be targeted to specific cells or receptors by any of the methods described herein. The actual dosage of reagent, formulation or composition that modulates a microbial, bacterial, viral or fungal disorder depends on many factors, including the size and health of an organism, however one of ordinary skill in the art can use the following teachings describing the methods and techniques for determining clinical dosages (Spilker B., *Guide to Clinical Studies and Developing Protocols*, Raven Press Books, Ltd., New York, 1984, pp. 7-13, 54-60; Spilker B., *Guide to Clinical Trials*, Raven Press, Ltd., New York, 1991, pp. 93-101; Craig C., and R. Stitzel, eds., *Modern Pharmacology*, 2d ed., Little, Brown and Co., Boston, 1986, pp. 127-33; T. Speight, ed., *Avery's Drug Treatment: Principles and Practice of Clinical Pharmacology and Therapeutics*, 3d ed., Williams and Wilkins, Baltimore, 1987, pp. 50-56; R. Tallarida, R. Raffa and P. McGonigle, *Principles in General Pharmacology*, Springer-Verlag, New York, 1988, pp. 18-20) to determine the

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appropriate dosage to use; but, generally, in the range of about 0.1 mg/kg to 1000 mg/kg, more specifically between about 1.0 mg/kg and 500 mg/kg, and preferably from about 10 mg/kg and 100 mg/kg inclusive final concentration are administered per day to an adult in any pharmaceutically-acceptable carrier.

5       The peptides of the present invention can also be used to treat an LPS associated disorder. With reference to an LPS associated disorder, the term "therapeutically effective amount" as used herein for treatment of an LPS associated disorder such as endotoxemia or sepsis refers to the amount of BGP-A or MGP-A peptide sufficient to decrease the subject's response to LPS and decrease the symptoms of an LPS associated  
10 disorder, such as sepsis. The term "therapeutically effective" therefore includes that the amount of BGP-A or MGP-A peptide sufficient to prevent, and preferably reduce by at least 50%, and more preferably sufficient to reduce by 90%, a clinically significant increase in the plasma level of LPS. The dosage ranges for the administration of BGP-A or MGP-A peptide are those large enough to produce the desired effect. Generally,  
15 the dosage will vary with the age, condition, sex, and extent of the infection with bacteria or other agent as described above, in the patient and can be determined by one skilled in the art. The dosage can be adjusted by the individual physician in the event of any contraindications. In any event, the effectiveness of treatment can be determined by monitoring the level of LPS or LPS associated molecules, such as tumor necrosis  
20 factor (TNF), in a patient. A decrease in serum LPS and TNF levels correlates positively with amelioration of the LPS associated disorder.

In a further embodiment, the present invention may be used as a food preservative or in treating food products to eliminate potential pathogens. The latter use might be targeted to the fish and poultry industries that have serious problems with enteric  
25 pathogens which cause severe human disease. In another embodiment, BGP-A or MGP-A may be used as disinfectants, for use in any product that must remain microbial free. In a further embodiment, BGP-A or MGP-A may be used as antimicrobials for food crops, either as agents to reduce post harvest spoilage, or expressed transgenically to enhance host resistance. Because of the antibiotic, antimicrobial, and antiviral properties  
30 of the peptides, they may also be used as preservatives or sterillants of materials

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- susceptible to microbial or viral contamination. The BGP-A or MGP-A peptides of the invention can be utilized as broad spectrum antimicrobial agents directed toward various specific applications. Such applications include use of the peptides as preservatives in processed foods (organisms including Salmonella, Yersinia, Shigella), either alone or in  
5 combination with antibacterial food additives such as lysozymes; as a topical agent (Pseudomonas, Streptococcus) and to kill odor producing microbes (Micrococci). The relative effectiveness of the peptides of the invention for the applications described can be readily determined by one of skill in the art by determining the sensitivity of any organism to one of the peptides.
- 10 It is also possible to incorporate the peptides on devices or immaterial objects where microbial growth is undesirable as a method of microbicidal inhibition or microbistatic inhibition of microbial growth in an environment capable of sustaining microbial growth by administering to the devices or immaterial objects a microbicidal or microbistatical effective amount of peptide. Such devices or immaterial objects include, but are not  
15 limited to, linens, cloth, plastics, implantable devices (e.g., heart pacemakers, surgical stents), surfaces or storage containers. Coating may be achieved by nonspecific absorption or covalent attachment.

### **EXAMPLES**

- The following examples are intended to illustrate but not admitted to limit the invention  
20 in any manner, shape, or form (either explicitly or implicitly), nor should they be so construed. While they are typical of those that might be used, other procedures, methodologies, or techniques known to those skilled in the art may be used alternatively.

## MATERIALS AND METHODS

*Bovine neutrophils.* Polymorphonuclear leukocytes (PMN) were purified from 1 L batches of fresh citrated bovine blood. Following sedimentation at 40 minutes at 700 x g and 37° C, the erythrocyte column was subjected to 7 seconds of hypotonic lysis, after which isotonicity was restored using 3x phosphate buffered saline. The leukocyte-rich suspension was then sedimented at 120 x g (4° C, 15 minutes). Residual erythrocytes were lysed by repeating this procedure 1 or 2 times. Aliquots were removed for quantitation by hemocytometry and differential counts. Preparations obtained by this procedure contained an average of  $4 \times 10^9$  cells per L of whole blood of which  $97 \pm 3\%$  were neutrophils. Preparations were treated with 2 mM diisopropylfluorophosphate (DFP). Neutrophil preparations were then cooled to 4° C for 20 minutes and disrupted by nitrogen cavitation in a Parr bomb (Borregaard, N., *et al.*, *J. Cell Biol.* 89:52-61, 1983). The cavitate was centrifuged at 800 x g for 10 minutes at 4° C, and the granule-containing supernatant was collected. Granules were harvested by centrifugation at 27,000 x G for 40 minutes and stored at -80° C.

*PMN Granule extracts.* Preparations of frozen granules from  $1-5 \times 10^{10}$  PMN were extracted with 5 ml of ice cold 10% acetic acid per  $1 \times 10^9$  cell equivalents. After stirring on ice for 18 hours, the suspension was clarified by centrifugation at 27,000 x G for 20 minutes at 4° C and the supernatants were lyophilized and stored at -70° C.

*Size exclusion chromatography.* Lyophilized granule extract was dissolved in 10% acetic acid at a concentration of ca.  $1 \times 10^9$  cell equivalents per ml, clarified by centrifugation, and loaded onto a 4.8 x 110 cm column of BioGel P-60 equilibrated in 5% acetic acid. The column was run at 8° C with an elution rate of 2 cm per hour, and 15 ml fractions were collected with continuous monitoring at 280 nm.

*Reversed phase HPLC (RP-HPLC).* Low molecular weight components eluting from the size exclusion column were further resolved by RP-HPLC on a Waters 510 binary system on a 1 x 25 cm Vydac C-18 column. Water and acetonitrile containing 0.1%

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trifluoroacetic acid (TFA) or 0.13% heptafluorobutyric acid (HFBA) were used for gradient elution. Purified peptides were lyophilized, dissolved in 0.01% acetic acid at 100 - 500 µg/ml, and stored at -70° C.

*Polyacrylamide gel electrophoresis.* Sodium dodecyl sulfate (SDS; 14) and acid-urea  
5 (Selsted, M.E., *et al.*, *Anal. Biochem.* 155:270-274, 1986) gel electrophoresis were used to the estimate molecular mass and/or purity of protein preparations as previously described (Selsted, M.E., *et al.*, *Infect. Immun.* 45:150-154, 1984).

*Amino acid analysis.* The amino acid composition of each peptide was determined on  
6 N HCl hydrolysates (2 h, 150° C) of native and performic acid-oxidized, or reduced  
10 and alkylated samples (Bidlemeier, B.A., *et al.*, *J. Chromatogr.* 336:93-104, 1984). Tryptophan content was determined by sequence analysis and by spectroscopic measurement on a Beckman DU 60 spectrophotometer by the method of Edelhock (Edelhock, H., *Biochem.* 6:1948-1954).

*Sequence Analysis.* For sequence analysis, purified BGP-A was subjected to automated  
15 Edman sequence analysis. Automated sequence analysis was performed on an Applied Biosystems 475A instrument configured with on-line PTH-amino acid analysis. The sequence was confirmed by comparing the primary structure with the amino acid composition, and cDNA cloning.

*Peptide synthesis.* BGP-A and BGP-A-amide were synthesized at the 0.4 mmol scale  
20 on a Millipore 9050 automated synthesizer by standard Fmoc/BOP/HOBt/NMM activation with a 30 minute coupling time. The starting resin for the free acid peptide was Fmoc-L-Valine-PEG-PS (Millipore), and for peptide amide the starting resin was Fmoc-PAL-PEG-PS (Barany, G., *et al.*, *Intercept*, R. Epton, Andover, England, 1992, pp.29-38; Van Abel, R.J., *et al.*, *Int. J. Peptide Protein Applicant respectfully requests*  
25 *withdrawal of the rejection.* 45:401-409, 1995). Side chain protecting groups were Pmc for arginine, trityl for glutamine and histidine, tBoc for lysine and tBu for tyrosine. Fmoc deprotection was with 2% piperidine and 2% DBU for 15 minutes. Tryptophan

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and isoleucine were double coupled. Following chain assembly the resin was cleaved and deprotected with reagent K (82.5% TFA, 5% phenol, 5% thioanisole, 5% water and 2.5% ethanedithiol) for 4 hours. The peptide solution was made 30% in acetic acid, extracted with dichloromethane, and the aqueous phase was lyophilized. Purification  
5 was performed by RP-HPLC on a 22.5 x 250 mm preparative Vydak C-18 column using 0.1%TFA and a linear acetonitrile gradient developed at 0.33% per minute. The purified peptides were analyzed by amino acid analysis, acid-urea gel electrophoresis and analytical RP-HPLC.

*cDNA isolation and characterization. BGP-A:* Total RNA was isolated from bovine  
10 bone marrow using the acid guanidinium thiocyanate-phenol extraction method of Chomczynski and Sacchi (Chomczynski, P., *et al.*, *Analyt. Biochem.* 162:156-159, 1987). Bone marrow total RNA (1 mg) was then used with avian reverse transcriptase to synthesize first strand cDNA according to the manufacturer's protocol (5'-RACE System; Life Technologies; Gaithersburg, MD). This cDNA was used as a template for  
15 3'-RACE, in which a degenerate gene specific primer was paired with an oligo (dT)<sub>15</sub>-anchor primer to generate the 3'-end of the BGP-A cDNA. PCR amplification was carried out using the following cycling parameters: 95 °C, 1 minutes; 55 °C, 1 minutes; 72 °C, 1 minutes for 35 cycles. 5'-RACE was carried out in a similar fashion with the exception that first strand cDNA was tailed using terminal transferase and different gene  
20 specific and anchor primers were used. PCR-amplified RACE products were subcloned and sequenced as described previously (Yount, N.Y., *et al.*, *J. Immunol.* 155:4476-4484, 1995). Once the 5'- and 3'-ends of the BGP-A cDNA were known, a PCR product corresponding to the full length BGP-A sequence was generated and characterized by sequence analysis.

25 Murine bone marrow total RNA and first strand cDNA were generated as for BGP-A. Two gene specific primers were then used to PCR amplify a sequence corresponding to a BGP-A homolog. This sequence was subcloned and sequenced as described above.

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*Antimicrobial assay.* *E. coli* ML35, *S. aureus* 502A, *C. albicans*, and *C. neoformans* were used as target organisms in a microbicidal suspension assay as previously described (Selsted, M.E., *Genetic Engineering: Principles and Methods*, J.K. Setlow, Plenum Press, New York, 1993, pp. 131-147).

## 5 EXAMPLE 1

### PURIFICATION OF BGP-A

- Previous electrophoretic analyses of the acid-soluble proteins of bovine PMN granules demonstrated that these preparations contain a complex mixture of proteins varying in size from 1,000 to 200,000 D (Selsted, M.E., *et al.*, *J. Biol. Chem.* 267:4292-4295, 10 1992). Acetic acid extract of a granule-enriched fraction from  $1.3 \times 10^{10}$  neutrophils was chromatographed on a Bio-Gel P-60 column as described above in the section titled, "Materials and Methods." Approximately  $2 \times 10^{10}$  cell equivalents of acid solubilized granule protein was fractionated on a BioGel P-60 column and antibacterial activity in pooled eluent fractions was assayed as described in the "Materials and Methods."
- 15 Fractions corresponding to Peak E were lyophilized and subjected to further purification by RP-HPLC. Each peak (A-F in Fig. 1A) contained bactericidal activity against *S. aureus* and *E. coli*. Peak F was predominantly comprising indolicidin, a novel thirteen residue antibiotic peptide amide (Selsted, M.E., *et al.*, *J. Biol. Chem.* 267:4292-4295), and Peak E contained at least 13  $\beta$ -defensins.
- 20 Peak E fractions were combined and further purified by HPLC. One tenth of the pooled fractions from Peak E (Fig. 1a) was loaded on a 1 x 25 cm Vydac C-18 column equilibrated in 0.1% TFA/water (solvent A) at a flow rate of 3.0 ml/min. A linear gradient of acetonitrile (20% to 45%) containing 0.1% TFA (solvent B) was applied at the rate of 0.33% per min. Fractions were collected using the peak cutting mode of a
- 25 Pharmacia Frac-200 fraction collector. The initial RP-HPLC purification of Peak E fractions yielded a complex chromatogram (Fig. 1B) in which most peaks contained two

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or more peptides as determined by acid-urea PAGE. However, BGP-A was eluted as an isolated, virtually pure peak (indicated by the asterisk symbol "\*" in Fig. 1B) early in the RP-HPLC chromatogram. Final purification (Fig. 2) was obtained by a second round of RP-HPLC.

## 5 EXAMPLE 2

### AMINO ACID AND SEQUENCE ANALYSIS OF BGP-A

The composition of BGP-A was established by amino acid analysis (Figure 2). Approximately 5 µg of purified BGP-A was injected onto a 0.4 x 25 cm Vydac C-18 column run at a flow rate of 1.0 ml/min. Solvents are the same as described above for Figure 1B. Gradient conditions: 10% B to 50% B in 25 min. *B. Acid-urea gel of purified BGP-A.* A 2 µg sample of purified BGP-A was loaded onto a 12.5% acid-urea polyacrylamide gel that was electrophoresed for 4 hours at 250 V (lane 2). A 100 µg sample of crude acid extract from bovine neutrophil granules (lane 1) was run in parallel. Staining was with Coomassie Blue containing 15% formalin. Absorbance scans of BGP-A were carried out between 300 and 200 nm, providing an accurate estimate of tyrosine and tryptophan content (Edelhoch, H., *Biochem.* 6:1948-1954, 1967). Automated sequence analysis was carried out on 2 nmol of BGP-A. Repetitive sequencing yields averaged ≥90 percent, allowing for unambiguous assignment of all thirteen residues. The complete amino acid sequence of BGP-A is:

20 Tyr-Lys-Ile-Ile-Gln-Gln-Trp-Pro-His-Tyr-Arg-Arg-Val (SEQ ID NO: 5; Fig. 6)

A protein sequence search using the BLAST algorithm (Altschul, S.F., *et al.*, *J. Molec. Biol.* 215:403-410, 1990) revealed no similar amino acid sequences among the GenBank Data base.

## EXAMPLE 3

### 25 SYNTHESIS OF BGP-A AND BGP-A-AMIDE

The two synthetic BGP-A forms were assembled as N<sup>α</sup>-Fmoc protected amino acids. (The acid-urea gel patterns of the purified peptides are shown in Fig. 3.) A 12.5% acid-urea gel was loaded with 2-4 µg of natural BGP-A (Fig. 3, lane 1), synthetic BGP-A (Fig. 3, lane 2) or synthetic BGP-A-amide (Fig. 3, lane 3). Staining was as described

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for Fig. 2. The yields of the HPLC-purified material were 31.4% for the free acid form, and 22.1% for the carboxamidated form.

#### **EXAMPLE 4**

##### **ISOLATION AND SEQUENCING OF BGP-A cDNA CLONES**

- 5 The full length BGP-A cDNA is 688 nucleotides in length (SEQ ID NO: 2) and predicts a 21 kD precursor composed of 190 residues (Fig. 4; SEQ ID NO: 3). Within the BGP-A precursor, 11 of the first 21 residues are hydrophobic and predict a signal peptide (Von Heijne, G., *Eur. J. Biochem.* **133**:17-21, 1983). The signal peptide domain is followed by an intervening propeptide region containing 156 residues. The final 13  
10 residues of the precursor correspond to the mature BGP-A peptide sequence (SEQ ID NO: 6).

- To determine if the BGP-A precursor was homologous to other nucleotide or protein sequences, a Blast search of the GenBank database was carried out. Some homology between the BGP-A sequence and a partial cDNA sequence isolated from murine  
15 adenocarcinoma of unknown tissue origin was identified. Using consensus primers derived from the murine adenocarcinoma and BGP-A sequences, a cDNA encoding a BGP-A like sequence from mouse bone marrow (Fig. 5; SEQ ID NO: 5) was isolated. This full-length cDNA is 679 nucleotides in length (SEQ ID NO: 4) and predicts a precursor comprising signal pro-peptide domains similar to those described for BGP-A  
20 (Fig. 5; SEQ ID NO: 5). The mature peptide sequence predicted by the murine cDNA is identical to BGP-A at 7 of 13 residues (Fig. 6; SEQ ID NO: 7). Based on this similarity, this sequence isolated from murine bone marrow cDNA is designated as mouse granulocyte peptide A (MGP-A; Fig. 5; SEQ ID NO: 5 and Fig. 6, SEQ ID NO: 7).

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**EXAMPLE 5****ANTIMICROBIAL ACTIVITY OF BGP-A AND BGP-A-AMIDE.**

Natural and synthetic BGP-A and synthetic BGP-A-amide were tested for their microbicidal activities against *S. aureus* 502A, *E. coli* ML35, *C. albicans*, and *C. neoformans*. Using a microbicidal suspension assay (Selsted, M.E., *Genetic Engineering: Principles and Methods*, J.K. Setlow, Plenum Press, New York, 1993, pp.131-147), each peptide was tested against the four test organisms with peptide concentrations ranging from 5-100 µg/ml. The bactericidal and fungicidal activities of the three peptide preparations were assessed using a standard microbicidal assay.

Organisms were grown to mid-log phase, harvested, and suspended to  $2 \times 10^7$  CFU/ml. The incubation mixture contained  $1-2 \times 10^6$  CFU/ml, 10 mM sodium phosphate buffer, pH 7.4, and peptide at concentrations up to 100 µg/ml. After 1 h of incubation at 37 C (4 h incubations for *C. neoformans*), serial 10-fold dilutions were plated on Trypticase Soy Agar (bacteria) or *S. abaroud* dextrose agar (fungi), and incubated for 24-48 h at 37 C. Killing was quantitated by colony counting, and plotted as a function of peptide concentration in the incubation.

The data, presented in Figure 7, reveal the dose-dependent activity of each peptide as measured by the reduction in colony forming units after a 1 or 4 hour incubation interval. These data demonstrate 1) that BGP-A was microbicidal against each organism; 2) that synthetic BGP-A and natural BGP-A were equal in potency, suggesting that the activity of the natural peptide was attributable to the purified compound and not to a contaminant; and 3) that the carboxamidated form of BGP-A is much more potent against most of the targets than is the free-carboxyl form.

The mature peptide was microbicidal *in vitro* against representative Gram positive and Gram negative bacteria, and yeast forms of two fungi. The antimicrobial activity of the natural peptide was validated by demonstration that synthetic BGP-A had equivalent killing activity.

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**EXAMPLE 6****ACTIVITY OF BGP-A AND BGP-A-AMIDE TO TREAT AN LPS DISORDER**

The effect of the BGP, MGP, BGP-A and MGP-A peptides of the invention on LPS-induced TNF in macrophages can be determined by those in the art, according to standard methods. For example, macrophage cells are grown by seeding cells into a cell culture flask and incubated at 37°C, 5% CO<sub>2</sub> for 1 week. Macrophage cell media [(Dulbecco's Modified Eagle Medium with Hepes buffer 450 ml; 2.4mM L-glutamine 3ml (400mM); Pen/Strep 3ml (10<sup>4</sup>U/ml of Pen, 1 mg/ml strep); and 10% heat inactivated fetal bovine serum (FBS) 50ml)] is then completely removed from flasks.

10 10 mls of cell dissociation solution (Sigma) is added to each flask and incubated at 37°C for 10 minutes. Cells are removed from flasks, diluted in macrophage cell media and centrifuged for approximately six minutes. The cell pellet is resuspended in 5ml of media/ flask used. 100µl cell suspension is removed and added to 400µl of trypan blue and cells are counted using a hemocytometer. The cell suspension is diluted to 1 x 10<sup>6</sup>

15 cells / ml and 1 ml of suspension is added per well of a 24 well plate. The 24 well plates are incubated at 37°C, 5% CO<sub>2</sub> overnight.

After an overnight incubation, the media is aspirated from all the wells. 100µl of Lipopolysaccharide (LPS) is added at 100ng/100µl. BGP-A and MGP-A is added at the desired concentration/100µl to specified wells. Macrophage cell media is added to a

20 final volume of 1 ml/well. The plates are incubated for six hours at 37°C, 5% CO<sub>2</sub>. The supernatant is removed from the wells and stored overnight at 4°C. For those wells in which whole bacteria is added directly to the wells, the supernatant is centrifuged in 0.2µm filter eppendorf tubes for 5 minutes.

The supernatants are then used in cell cytotoxic L929 assay. The samples are

25 transferred to 96 well plates. 50µl of TNF media is added to all the wells in all the plates except to those wells in the first row. 10µl of murine TNF standard (20ng/ml) and 90µl of TNF media is added in duplicate to the plate and diluted 1:2 down the plate to the second to last row. Test samples (75µl), comprising the supernatants from the

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macrophage cell assays, are added to separate rows in duplicate and diluted 1:3 to the second to last rows.

TNF-sensitive L929 mouse fibroblast cells are grown by seeding  $10^6$  cells into a  $162\text{cm}^2$  cell culture flask and left to grow for 1 week. L929 cells are removed from the flask with 10mls of trypsin-EDTA/flask and incubated 3-5 minutes. Cell suspension is diluted and centrifuged for 6 minutes. The pellet is resuspended in 5 mls of fresh L929 media/flask and counted (same as macrophage cells). Cell suspension is diluted to  $10^6$  cells/ml. 100 $\mu$ l is used to inoculate each well of the 96 well plates with the supernatants. (L929 Growth Media is the same as macrophage cell media except instead of FBS, 50 mls of 10% heat inactivated horse serum is utilized; TNF Assay Media is the same as macrophage cell media except 4 $\mu$ g/ml Actinomycin D is added.)

The plates are incubated at 37°C at 5% CO<sub>2</sub> for 2 days. The media is then aspirated and replaced with 100 $\mu$ l of the dye MTT (0.5mg/ml) in modified Eagle Medium without phenol red. The plates are then incubated at 37°C at 5% CO<sub>2</sub> for 3 hours. The dye is then removed and replaced with 100 $\mu$ l of absolute ethanol. The plates are left at room temperature for 10 - 15 minutes to dissolve the formazan dye crystals.

The plates are read at 570nm in a ELISA plate reader with 690nm reference filter. One unit of TNF activity is defined as the amount required to kill 50% of the L929 cells. The TNF level in Units per ml therefore is the reciprocal of the dilution which led to a 50% killing of L929 cells.

It is to be understood that, while the invention has been described with reference to the above detailed description, the foregoing description is intended to illustrate, but not to limit, the scope of the invention. Other aspects, advantages, and modifications of the invention are within the scope of the following claims. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

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## CLAIMS

What is claimed is:

1. An isolated antimicrobial peptide comprising an amino acid sequence YXXIQXWXHYR (SEQ ID NO: 1), wherein X can be any amino acid.
- 5 2. The peptide of claim 1, wherein the amino acid sequence is set forth in SEQ ID NO: 6.
3. The peptide of claim 1, wherein the amino acid sequence is set forth in SEQ ID NO: 7.
4. The peptide of any of claims 1-3, wherein the peptide comprises at least  
10 one modified amino acid.
5. The peptide of claim 4, wherein the modified amino acid comprises a carboxy terminal amide.
6. The peptide of claim 1, wherein the peptide exhibits antimicrobial activity against microorganisms selected from the group consisting of  
15 gram positive bacteria, gram negative bacteria, fungi and viruses.
7. The peptide of claim 6, wherein the organism is selected from the group consisting of: *S. aureus*, *E. coli*, *C. albicans*, *S. typhimurium*, and *C. neoformans*.
8. An isolated antimicrobial polypeptide having an amino acid sequence as  
20 set forth in SEQ ID NO: 3 or functional fragments thereof.

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9. An isolated antimicrobial polypeptide having an amino acid sequence as set forth in SEQ ID NO: 5 or functional fragments thereof.
10. An isolated nucleic acid sequence encoding the peptide of SEQ ID NO: 1 or functional fragments thereof.
- 5 11. An isolated nucleic acid sequence encoding the peptide of SEQ ID NO: 6 or functional fragments thereof.
12. An isolated nucleic acid sequence encoding the peptide of SEQ ID NO: 7 or functional fragments thereof.
13. An isolated nucleic acid sequence encoding the polypeptide of SEQ ID  
10 NO: 3 or functional fragments thereof.
14. An isolated nucleic acid sequence encoding the polypeptide of SEQ ID NO: 5 or functional fragments thereof.

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15. The polynucleotide of claims 13 or 14, wherein such sequence is characterized by:
- a) nucleotide sequences which hybridize under stringent conditions with the polynucleotide of claim 13 or 14;
  - b) nucleotide sequences which encode peptides with conservative variations from the amino acid sequences encoded by the DNA of claim 13 or 14;
  - c) the nucleotide sequence of claim 13 or 14, wherein T is U;
  - d) functional fragments of a), b), or c) which encode peptides which retain the biological activity of BGP-A, or MGP-A; and
  - e) degenerate nucleotide sequences encoding the amino acid sequence as encoded by any of a), b), c) or d).
16. An antibody that binds to SEQ ID NO: 1.
17. The antibody of claim 16, wherein the antibody is monoclonal.
18. The antibody of claim 16, wherein the antibody is polyclonal.
19. A method of microbicidal or microbistatic inhibition in an environment capable of sustaining microbial growth comprising administering to the environment a microbicidal or microbistatcal effective amount of a peptide having an amino acid sequence of YXXIQXWXHYR (SEQ ID NO: 1), wherein X can be any amino acid.

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20. The method of claim 19, wherein the peptide has the amino acid sequence set forth in SEQ ID NO: 6.
21. The method of claim 19, wherein the peptide has the amino acid sequence set forth in SEQ ID NO: 7.
- 5 22. The method of claim 19, wherein the peptide has the amino acid sequence set forth in SEQ ID NO: 3, or functional fragments thereof.
23. The method of claim 19, wherein the peptide has the amino acid sequence set forth in SEQ ID NO: 5, or functional fragments thereof.
24. The method of claim 19, further comprising at least one additional  
10 antimicrobial composition.
25. The method of claim 24, wherein the antimicrobial composition is selected from the group consisting of an antibiotic, an antifungal, and an antiviral agent.
26. The method of claim 25, wherein the antibiotic agent is selected from  
15 a class of antibiotic agents selected from the group consisting of aminoglycosides, penicillins, cephalosporins, carbapenems, monobactams, quinolones, tetracyclines, glycopeptides, chloramphenicol, clindamycin, trimethoprim, sulfamethoxazole, nitrofurantoin, rifampin and mupirocin.
27. The method of claim 26, wherein the antibiotic agent is selected from the  
20 group consisting of amikacin, gentamicin, kanamycin, netilmicin, tobramycin, streptomycin, azithromycin, clarithromycin, erythromycin, erythromycin estolate/ethylsuccinate/glucaptate/lactobionate/stearate, penicillin G, penicillin V, methicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin, ampicillin, amoxicillin, ticarcillin, carbenicillin, mezlocillin,

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azlocillin, piperacillin, cephalothin, cefazolin, cefaclor, cefamandole, cefoxitin, cefuroxime, cefonicid, cefmetazole, cefotetan, cefprozil, loracarbef, cefetamet, cefoperazone, cefotaxime, ceftizoxime, ceftriaxone, ceftazidime, cefepime, cefixime, cefpodoxime, cefsulodin, imipenem, 5 aztreonam, fleroxacin, nalidixic acid, norfloxacin, ciprofloxacin, ofloxacin, enoxacin, lomefloxacin, cinoxacin, doxycycline, minocycline, tetracycline, vancomycin, and teicoplanin.

28. The method of claim 19, wherein the peptide comprises at least one modified amino acid.
- 10 29. The method of claim 28, wherein the modified amino acid comprises a carboxy terminal amide.
30. The method of claim 19, wherein the peptide is an effective microbicidal or microbistatic agent against microorganisms selected from the group consisting of gram positive bacteria, gram negative bacteria, 15 fungi and viruses.
31. The peptide of claim 30, wherein the organism is selected from the group consisting of: *S. aureus*, *E. coli*, *C. albicans*, *S. typhimurium*, and *C. neoformans*.
32. The method of claim 19, wherein the environment is an organism.
- 20 33. The method of claim 32, wherein the environment is an animal.
34. The method of claim 32, wherein the environment is a human.
35. The method of claim 19, wherein the environment is a food or food product.

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36. The method of claim 19, wherein the environment is a water supply.
37. A method of inhibiting a lipopolysaccharide (LPS) associated disorder in a subject having, or at risk of having, such a disorder, comprising administering to the subject a therapeutically effective amount of a peptide having an amino acid sequence selected from the group consisting of: SEQ ID NO: 1; SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6 and SEQ ID NO: 7 or functional fragments thereof.
38. The method of claim 37, further comprising at least one additional antimicrobial composition.
39. The method of claim 38, wherein the antimicrobial composition is selected from the group consisting of an antibiotic, an antifungal, and an antiviral agent.
40. The method of claim 39, wherein the antibiotic agent is selected from a class of antibiotic agents selected from the group consisting of aminoglycosides, penicillins, cephalosporins, carbapenems, monobactams, quinolones, tetracyclines, glycopeptides, chloramphenicol, clindamycin, trimethoprim, sulfamethoxazole, nitrofurantoin, rifampin and mupirocin.

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41. The method of claim 40, wherein the antibiotic agent is selected from the group consisting of amikacin, gentamicin, kanamycin, netilmicin, tobramycin, streptomycin, azithromycin, clarithromycin, erythromycin, erythromycin estolate/ethylsuccinate/glucaptate/lactobionate/stearate, penicillin G, penicillin V, methicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin, ampicillin, amoxicillin, ticarcillin, carbenicillin, mezlocillin, azlocillin, piperacillin, cephalothin, cefazolin, cefaclor, cefamandole, cefoxitin, cefuroxime, cefonicid, cefmetazole, cefotetan, cefprozil, loracarbef, cefetamet, cefoperazone, cefotaxime, ceftizoxime, ceftriaxone, ceftazidime, cefepime, cefixime, cefpodoxime, cefsulodin, imipenem, aztreonam, fleroxacin, nalidixic acid, norfloxacin, ciprofloxacin, ofloxacin, enoxacin, lomefloxacin, cinoxacin, doxycycline, minocycline, tetracycline, vancomycin, and teicoplanin.
42. The method of claim 37, wherein the peptide comprises at least one modified amino acid.
43. The method of claim 42, wherein the modified amino acid comprises a carboxy terminal amide.
44. A method of inhibiting protozoan growth comprising contacting a protozoan with an inhibitory effective amount of a peptide selected from the group consisting of SEQ ID NO: 1, 6 and 7.



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FIG. 1A

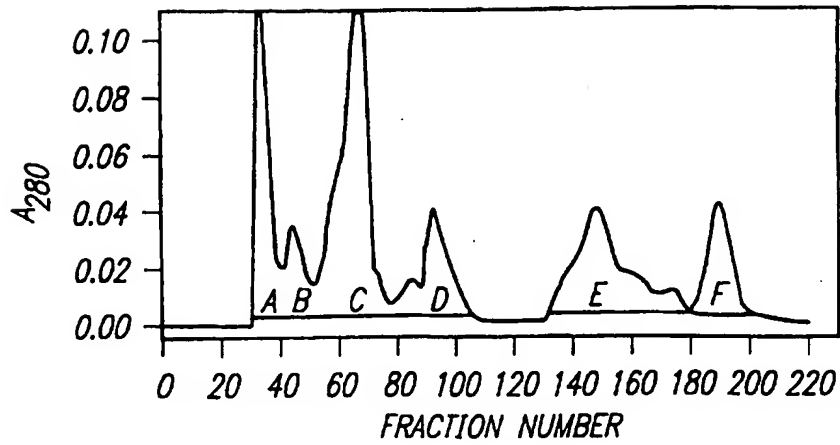


FIG. 1B

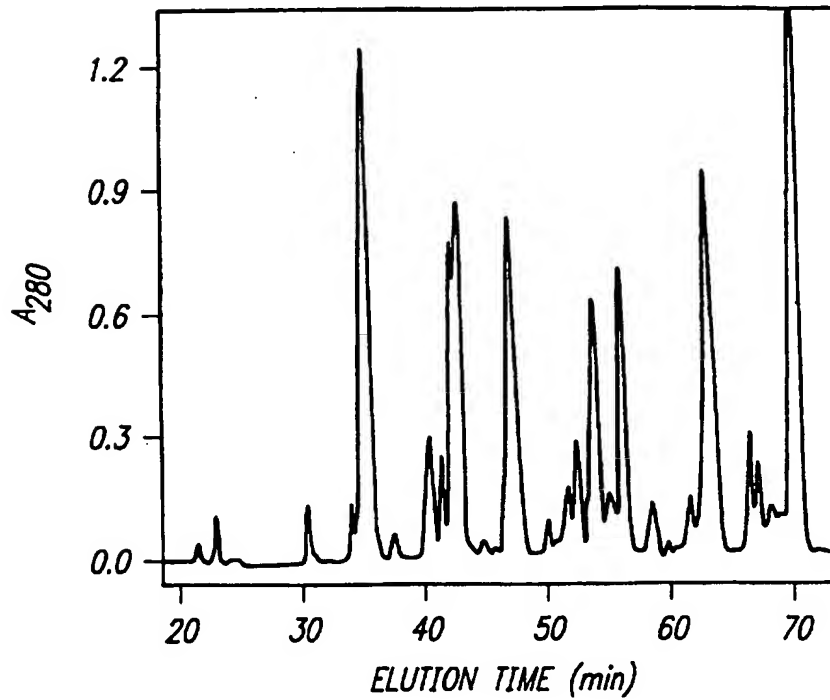


FIG. 6

BNP-A  
MNP-A

Y	K	I	I	Q	Q	W	P	H	Y	R	R	V
Y	Q	V	I	Q	S	W	E	H	Y	R	E	

13  
12



WO 97/29765

PCT/US97/02218

2/5

FIG. 2A

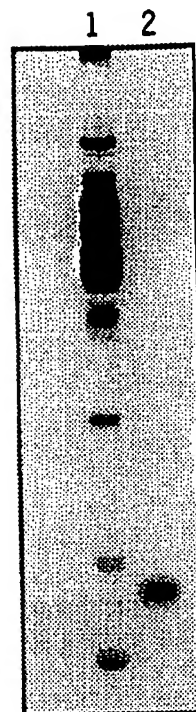
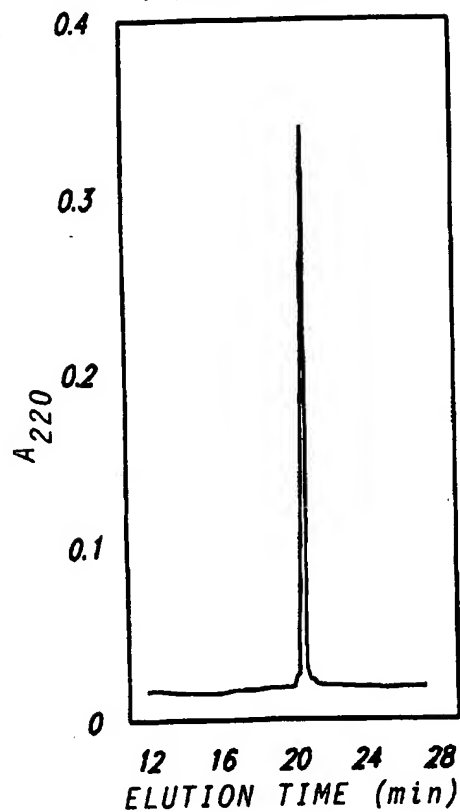
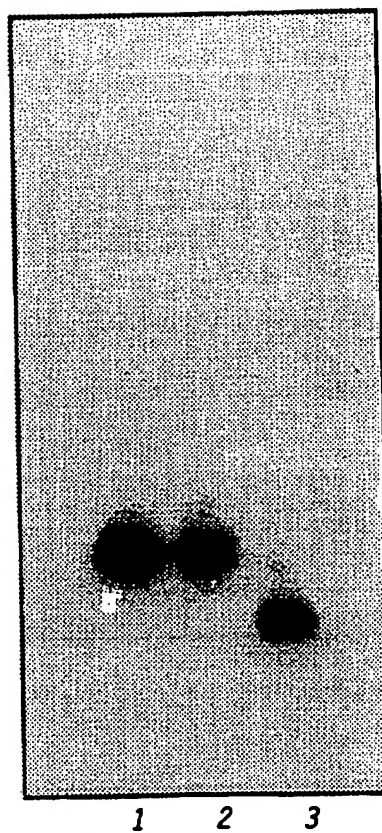


FIG. 2B

FIG. 3





3/5

AGTCTCCGCGTGTCTTTCTGCCTGCCATGTCTCGCCGCTACACACCGC	50
M S R R Y T P	
TCGCCTGGGTCTCTCTCGCCCTCTGGGCCTCGGGGCGGCTCAAGACTGC	100
L A W V L L A L L G L G A A Q D C	
GGCAGCATCGTGTCCCGCGGAAAGTGGGGCGCCCTGGCATCCAAGTGCAG	150
G S I V S R G K W G A L A S K C S	
CCAGAGGCTAAGACAGCCTGTGCGCTACGTGGTGGTGTGCGACACGGCGG	200
Q R L R Q P V R Y V V V S H T A	
GCAGCGTCTGCAACACTCCGGCCTCGTGCCAGAGGCAGGCCCAAAACGTG	250
G S V C N T P A S C Q R Q A Q N V	
CAGTACTACCACGTGCGGGAGCGGGCTGGTGCACGTGGGCTACAATTT	300
Q Y Y H V R E R G W C D V G Y N F	
CCTGATCGGAGAAGATGGGCTCGTGTATGAGGGCCGGGCTGGAACACCT	350
L I G E D G L V Y E G R G W N T	
TAGGTGCTCACTCTGGGCCCACGTGGAACCCCATAGCCATCGGCATCTCC	400
L G A H S G P T W N P I A I G I S	
TTCATGGGCAACTACATGCATCGGGTGCCCCGGGCTCTGCTCTCAGGGC	450
F M G N Y M H R V P P A S A L R A	
GGCCAGAGTCTGCTGGCTTGTGGCGCAGCTCGGGGATACCTGACTCCTA	500
A Q S L L A C G A A R G Y L T P	
ACTACGAAGTCAAAGGACACCGCGATGTGCAGCAGACGCTCTCTCCAGGG	550
N Y E V K G H R D V Q Q T L S P G	
GACGAGCTCTATAAAATCATCCAGCAGTGGCCGCACTACCGCCGCGTGTG	600
D E L <u>Y K I I Q Q W P H Y R R V</u>	
AGGGCCTGTCCGTCTTCTCACACCCACCCATCCCATCAGAAACCCACC	650
GCCTTCCCCTGCCCCAATAAAGGCGAAGCTTAAACTGT	688

FIG. 4



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ATACACAGCCCTGCGTCCTGTGCGGCACGTCCAGCATGTTGTTTGCCTGT	50
M L F A C	
GCTCTCCTTGCCCTCCTGGGTCTGGCAACCTCCTGCAGTTTCATCGTGCC	100
A L L A L L G L A T S C S F I V P	
CCGCAGTGAGTGGAGGGCCCTGCCATCCGAGTGCTCTAGCCGCCTGGGGC	150
R S E W R A L P S E C S S R L G	
ACCCAGTTCGCTACGTGGTGATCTCACACACAGCCGGCAGCTTCTGCAAC	200
H P V R Y V V I S H T A G S F C N	
AGCCCGGACTCCTGTGAACAGCAGGCCCGCAATGTGCAGCATTACCACAA	250
S P D S C E Q Q A R N V Q H Y H K	
GAATGAGCTGGGCTGGTGCGATGTAGCCTACAACCTTCCTTATTGGAGAGG	300
N E L G W C D V A Y N F L I G E	
ACGGTCATGTCTATGAAGGCCGAGGCTGGAACATCAAGGGTGACCACACA	350
D G H V Y E G R G W N I K G D H T	
GGGCCCATCTGGAATCCCATGTCTATTGGCATCACCTTCATGGGGAACCT	400
G P I W N P M S I G I T F M G N F	
CATGGACCGGGTACGCAAAGCGGCCCTCCGTGCTGCCCTAAATCTTCTG	450
M D R V R K A A L R A A L N L L	
GAATCTGGGGTGTCTCGGGGCTTCCTGAGATCCAACCTATGAAGTCAAAGG	500
E S G V S R G F L R S N Y E V K G	
ACACCGGGATGTGCAAAGCACTCTCTCTCCAGGTGACCAACTCTATCAGG	550
H R D V Q S T L S P G D Q L <u>Y Q</u>	
TCATCCAAAGCTGCGAACACTACCGAGAGTGAGAGACCTTGAGACCTAGT	600
<u>V I Q S W E H Y R E</u>	
GAGAATCCCCCCCCCAGCCCGAAATCCCTCCTGCCACCTGCTTCTTCC	650
CATTGACCCCCAATAAAGACTCAGCACC	678

FIG. 5



FIG. 7-1

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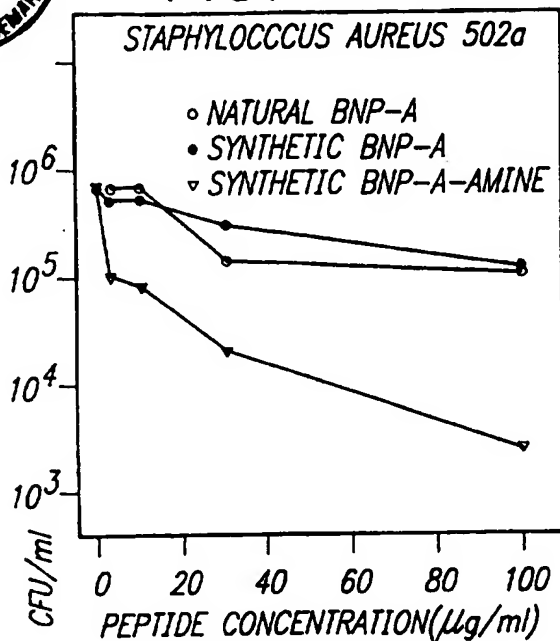


FIG. 7-2

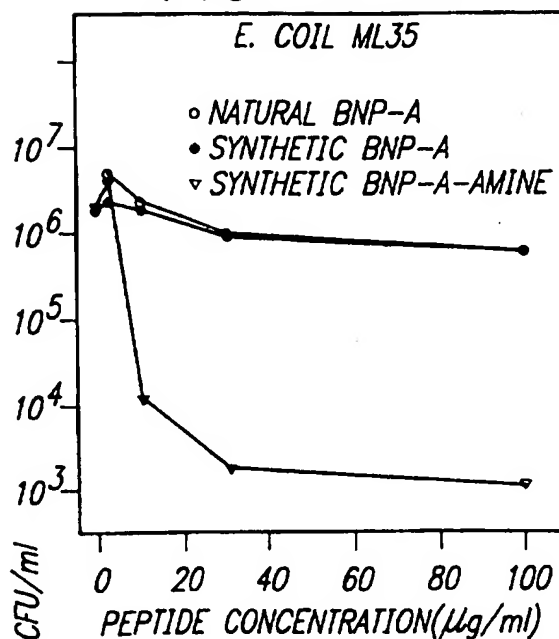


FIG. 7-3

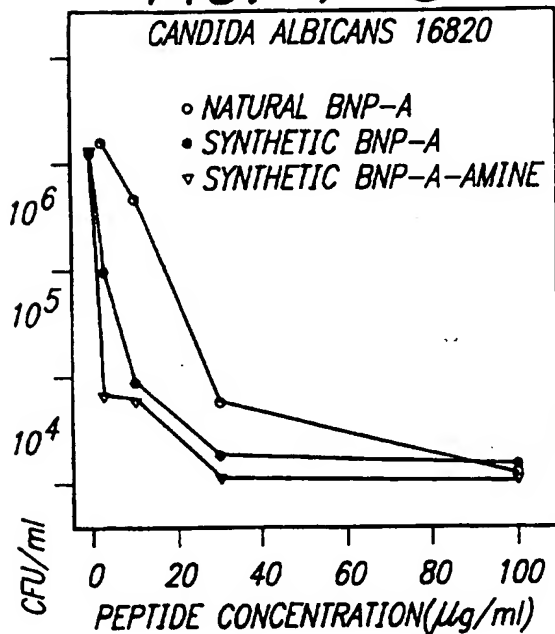
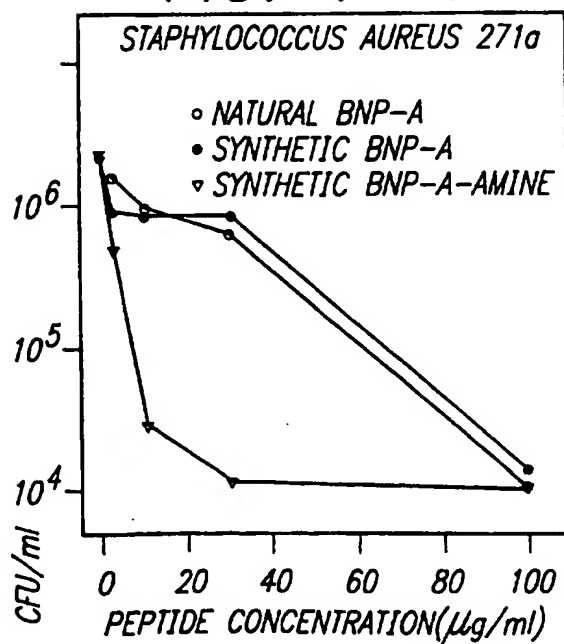
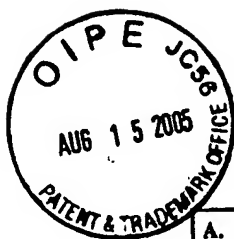


FIG. 7-4





## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/02218

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 38/00, 38/04, 39/12; C07K 5/00, 7/00, 16/00, 17/00

US CL : 424/185.1; 514/14, 15; 530/327, 388.24, 389.2; 536/23.5

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/185.1; 514/14, 15; 530/327, 388.24, 389.2; 536/23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, EMBASE, LIFESCI, REGISTRY, BIOSIS, WPID

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,459,235 A (SELSTED ET AL.) 17 October 1995, see entire document.	1-44
Y	DIAMOND et al. Airway epithelial cells are the site of expression of a mammalian antimicrobial peptide gene. Proc. Natl. Acad. Sci. USA. May 1993, Vol. 90, pages 4596-4600, see entire document.	1-44
Y	SELSTED et al. Defensins in granules of phagocytic and non-phagocytic cells. Trends in Cell Biology. March 1995, Vol. 5, pages 114-119, see entire document.	1-44

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z*	document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means		
*P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

07 APRIL 1997

Date of mailing of the international search report

15 APR 1997

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
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Authorized officer

PATRICK NOLAN

Telephone No. (703) 308-0196

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/02218

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ZANETTI et al. The cDNA of the Neutrophil Antibiotic Bac5 Predicts a Pro-sequence Homologous to a Cysteine Proteinase Inhibitor That Is Common to Other Neutrophil Antibiotics. J. of Biol. Chem. 05 January 1993, Vol. 268, No. 1, pages 522-526, see entire document.	1-44



Sequence 1: SEQ ID NO:3 from WO 97/29765 (bovine granulocyte peptide A precursor)

Sequence 2: SEQ ID NO:5 from WO 97/29765 (mouse granulocyte peptide A precursor)

Score = 188 bits (477), Expect = 1e-46

Identities = 100/180 (55%), Positives = 120/180 (66%), Gaps = 19/180 (10%)

Query: 9 AWWLLALLGLGAAQDCGSIVSRGKWGALASKCSQRLRQPVRYVVVSHTAGSVCNTPASCQ 68

A LLALLGL A C IV R +W AL S+CS RL PVRVYV+SHT GS CN+ SC+

Sbjct: 4 ACALLALLGL--ATSCSFIVFRSEWRALPSECSSRLGHPVRYVVVISHTRGSFCNSFDSCE 61

Query: 69 RQAQNVQYYHVRERGWCDVGYNFKIGEDGKVYEGRGWNTKGDHSGPTWNPIAIGISFMGN 128

+QA+NVQ+YH E WCDV YN K DH+ P +NP++IGI+FMGN

Sbjct: 62 QQARNVQHYHKNELEWCDVAYNI-----KEDHTEPIYNPMSIGITFMGN 105

Query: 129 YMHRVFFASALRAAQSLACGAARGYLTPNYEVKGHRDVQQTLSPGDELYKIIQQWPHYR 188

+M RV A ALRAA +LL G +RG+L NYEVKGHRDVQ LS GD+ Y++IQ W HYR

Sbjct: 106 FMDRVKA-ALRAALNLLESGVSRGFLRSNYEVKGHRDVQSFLSFGDQKYQVIQSWEHYR 164

CPU time: 0.02 user secs. 0.01 sys. secs 0.03 total secs.

Lambda	K	H
0.322	0.136	0.448

Gapped Lambda	K	H
0.267	0.0410	0.140

Matrix: BLOSUM62

Gap Penalties: Existence: 11, Extension: 1

Number of Sequences: 1

Number of Hits to DB: 300

Number of extensions: 136

Number of successful extensions: 3

Number of sequences better than 10.0: 1

Number of HSP's better than 10.0 without gapping: 1

Number of HSP's gapped: 1

Number of HSP's successfully gapped: 1

Number of extra gapped extensions for HSPs above 10.0: 0

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Length adjustment: 123

Effective length of query: 67

Effective length of database: 850,049,207

Effective search space: 56953296869

Effective search space used: 56953296869

Neighboring words threshold: 9

Window for multiple hits: 0

X1: 16 ( 7.4 bits)

X2: 129 (49.7 bits)

X3: 129 (49.7 bits)

S1: 41 (21.9 bits)

S2: 73 (32.7 bits)



US006696559B1

(12) **United States Patent**  
Selsted(10) Patent No.: **US 6,696,559 B1**  
(45) Date of Patent: **\*Feb. 24, 2004**(54) **ANTIMICROBIAL PEPTIDES AND METHODS OF USE**(75) Inventor: **Michael E. Selsted, Irvine, CA (US)**(73) Assignee: **The Regents of the University of California, Oakland, CA (US)**

(\*) Notice: This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/421,630**(22) Filed: **Oct. 19, 1999****Related U.S. Application Data**

(62) Division of application No. 08/799,149, filed on Feb. 14, 1997, now Pat. No. 6,008,195.

(60) Provisional application No. 60/011,834, filed on Feb. 16, 1996.

(51) Int. Cl.<sup>7</sup> ..... **C12N 15/12; C07K 7/08; C07K 14/47**(52) U.S. Cl. .... **536/23.5; 536/23.1; 435/320.1; 435/252.3; 435/69.1; 530/327; 530/350**(58) Field of Search ..... **536/23.1, 23.5; 435/320.1, 252.3, 69.1; 530/327, 350**(56) **References Cited****U.S. PATENT DOCUMENTS**

5,459,235 A 10/1995 Selsted et al. .... 530/300

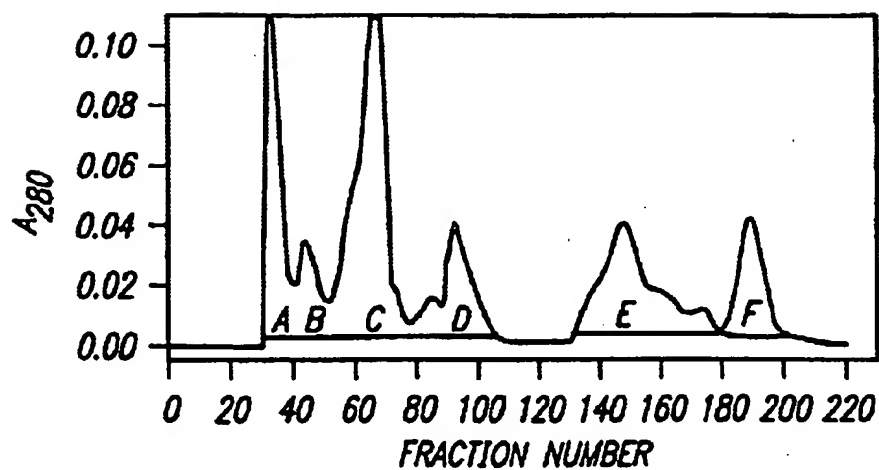
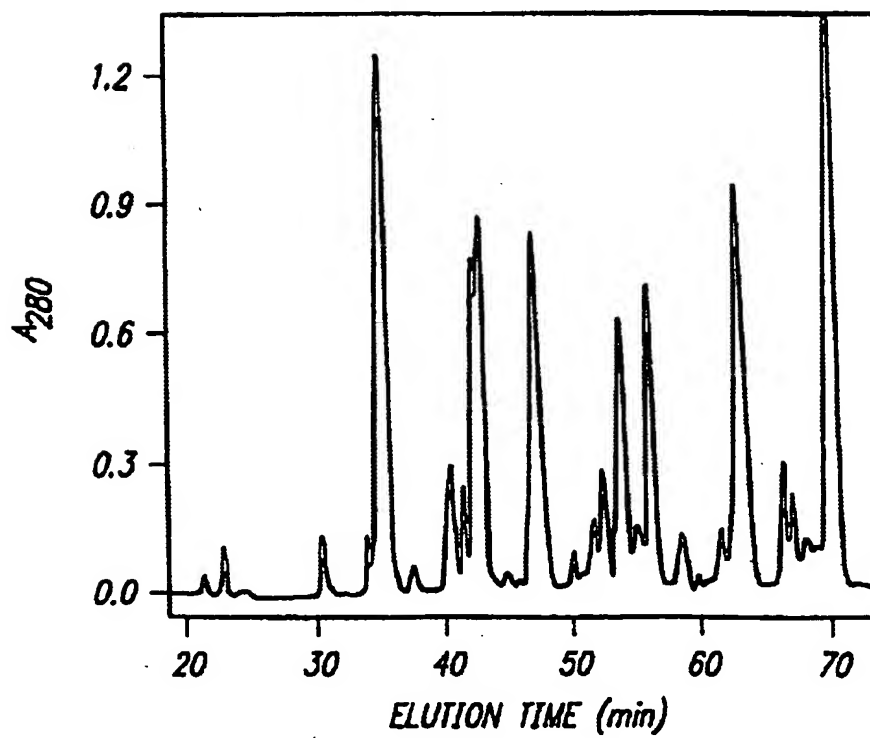
**FOREIGN PATENT DOCUMENTS**

WO WO 94/21672 9/1994

**OTHER PUBLICATIONS**Kustikova, O.S., "M. Musculus mRNA for TAG7 Protein," *Database EMBL 'Online'*, Database Accession No. X86374, Apr. 20, 1995, 2 pages.Larrick, J. W. et al., "Anti-Microbial Activity of Human CAP18 Peptides," *Immunotechnology*, vol. 1, No. 1, May 1, 1995, pp. 65-72.Diamond et al., "Airway epithelial cells are the site of expression of mammalian antimicrobial peptide gene", *Proc. Natl. Acad. Sci.*, vol. 90, pp. 4596-4600, May, 1993.Selsted et al., "Defensins in granules of phagocytic and non-phagocytic cells", *Trends in Cell Biology*, vol. 5, Mar. 1995.Zanetti et al., The cDNA of the Neutrophil Antibiotic Bac5 Predicts a Pro-sequence Homologous to a Cysteine Proteinase Inhibitor That Is Common to Other Neutrophil Antibiotics, *J. Of Biol. Chem.*, Jan. 5, 1993, vol. 268, No. 1, pp. 522-526.*Primary Examiner*—Rebecca E. Prouty(74) *Attorney, Agent, or Firm*—Gray Cary Ware & Freidenrich, LLP; Lisa A. Haile; Kelly K. Reynolds(57) **ABSTRACT**

Novel antimicrobial peptides from bovine and murine neutrophils are provided. The peptides, designated bovine granulocyte peptide A (BGP-A) and murine granulocyte peptide A (MGP-A) were purified to homogeneity from peripheral blood granulocytes. The amino acid and nucleotide sequence of BGP-A and MGP-A are also provided. A synthetic version of BGP-A and MGP-A is also provided. The purified BGP-A peptide is shown to have antimicrobial activity indistinguishable from that of natural BGP-A. Synthetic carboxamidated analogs of BGP-A (BGP-A-amide) and MGP-A (MGP-A-amide) are also provided.

**6 Claims, 6 Drawing Sheets****BGP-A****MGP-A****13****12**

*FIG. 1A**FIG. 1B*

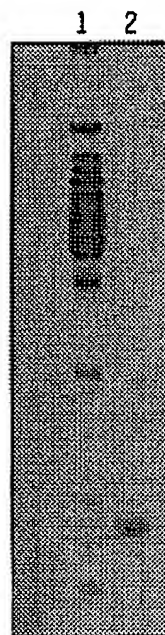
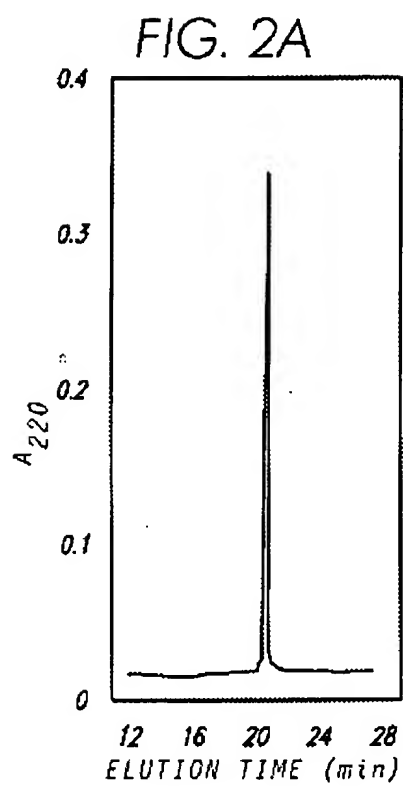
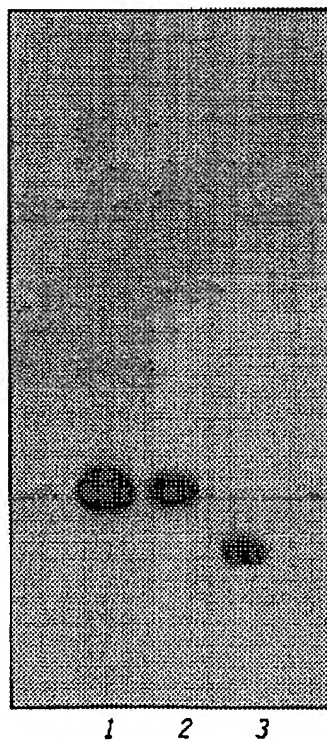


FIG. 2B

FIG. 3



AGTCTCCGCGTGTCTTTCCTGCCTGCCATGTCTCGCCGCTACACACCGC	50
M S R R Y T P	
TCGCCTGGGTCCTCCTCGCCCTCCTGGGCCCTCGGGGCGGCTCAAGACTGC	100
L A W V L L A L L G L G A A Q D C	
GGCAGCATCGTGTCCCGCGGAAAGTGGGGCGCCCTGGCATCCAAGTGCAG	150
G S I V S R G K W G A L A S K C S	
CCAGAGGCTAAGACAGCCTGTGCGCTACGTGGTGGTGTGCGACACGGCGG	200
Q R L R Q P V R Y V V V S H T A	
GCAGCGTCTGCAACACTCCGGCCTCGTGCCAGAGGCAGGCCCAAAACGTG	250
G S V C N T P A S C Q R Q A Q N V	
CAGTACTACCACGTGCGGGAGCGGGGCTGGTGCACGTGGGCTACAATT	300
Q Y Y H V R E R G W C D V G Y N F	
CCTGATCGGAGAAGATGGGCTCGTGTATGAGGGCCGGGCTGGAACACCT	350
L I G E D G L V Y E G R G W N T	
TAGGTGCTCACTCTGGGCCACGTGGAACCCCATAGCCATCGGCATCTCC	400
L G A H S G P T W N P I A I G I S	
TTCATGGGCAACTACATGCATCGGGTGCCCCGGCCTCTGCTCTCAGGGC	450
F M G N Y M H R V P P A S A L R A	
GGCCAGAGTCTGCTGGCTTGTGGCGCAGCTCGGGGATACCTGACTCCTA	500
A Q S L L A C G A A R G Y L T P	
ACTACGAAGTCAAAGGACACCGCGATGTGCAGCAGACGCTCTCTCCAGGG	550
N Y E V K G H R D V Q Q T L S P G	
GACGAGCTCTATAAAATCATCCAGCAGTGCCGCACTACCGCCGCGTGTG	600
D E L <u>Y K I I Q Q W P H Y R R V</u>	
AGGGCCTGTCCGTCTTCTCACACCCACCCATCCCATCAGAAACCCACC	650
GCCTTCCCCTGCCCAATAAAGGCGAAGCTTAACTGT	688

FIG. 4

ATACACAGCCCTGCGTCCTGTGCGGCACGTCCAGCATGTTGTTTGCCTGT	50
M L F A C	
GCTCTCCTTGCCCTCCTGGGTCTGGCAACCTCCTGCAGTTTCATCGTGCC	100
A L L A L L G L A T S C S F I V P	
CCGCAGTGAGTGGAGGGCCCTGCCATCCGAGTGCTCTAGCCGCCTGGGGC	150
R S E W R A L P S E C S S R L G	
ACCCAGTTCGCTACGTGGTGATCTCACACACAGCCGGCAGCTTCTGCAAC	200
H P V R Y V V I S H T A G S F C N	
AGCCCGGACTCCTGTGAACAGCAGGCCCGCAATGTGCAGCATTACCACAA	250
S P D S C E Q Q A R N V Q H Y H K	
GAATGAGCTGGGCTGGTGCGATGTAGCCTACAACCTCCTTATTGGAGAGG	300
N E L G W C D V A Y N F L I G E	
ACGGTCATGTCTATGAAGGCCGAGGCTGGAACATCAAGGGTGACCACACA	350
D G H V Y E G R G W N I K G D H T	
GGGCCCATCTGGAATCCCATGTCTATTGGCATCACCTTCATGGGGAACCT	400
G P I W N P M S I G I T F M G N F	
CATGGACCGGGTACGCAAAGCGGCCCTCCGTGCTGCCCTAAATCTTCTG	450
M D R V R K A A L R A A L N L L	
GAATCTGGGGTGTCTCGGGGCTTCCTGAGATCCAACTATGAAGTCAAAGG	500
E S G V S R G F L R S N Y E V K G	
ACACCGGGATGTGCAAAGCACTCTCTCTCCAGGTGACCAACTCTATCAGG	550
H R D V Q S T L S P G D Q L <u>Y Q</u>	
TCATCCAAAGCTGCGAACACTACCGAGAGTGAGAGACCTTGAGACCTAGT	600
<u>V I Q S W E H Y R E</u>	
GAGAATCCCCCCCCCAGCCCGAAATCCCTCCTGCCACCTGCTTCTTCC	650
CATTGACCCCCAATAAAGACTCAGCACC	678

FIG. 5

FIG. 6

BGP-A  
MGP-A

Y	K	I	I	Q	Q	W	P	H	Y	R	R	V
Y	Q	V	I	Q	S	W	E	H	Y	R	E	

13  
12

FIG. 7A

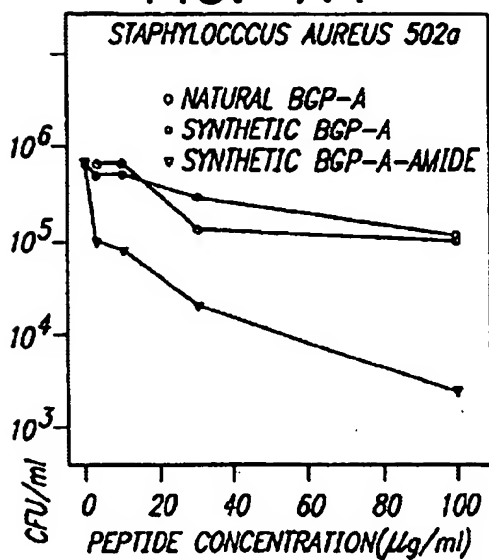


FIG. 7B

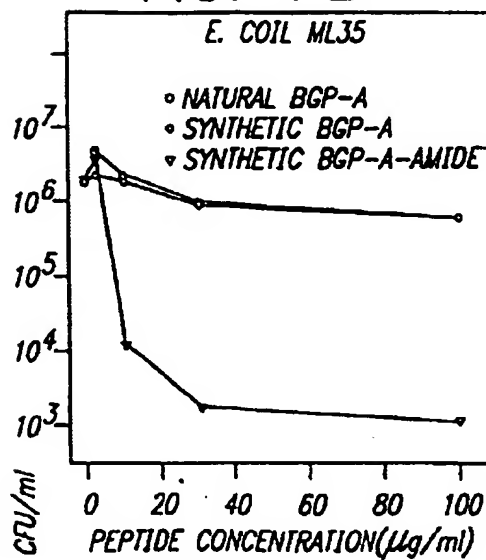


FIG. 7C

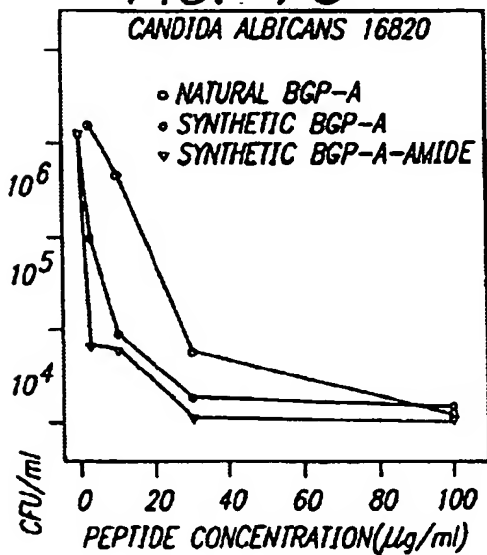
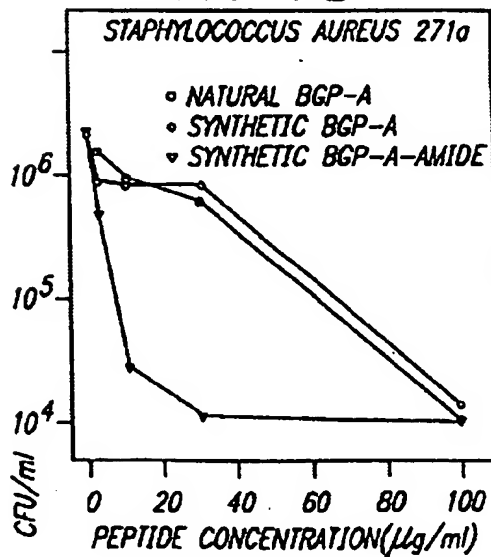


FIG. 7D



1

## ANTIMICROBIAL PEPTIDES AND METHODS OF USE

This application is a divisional of U.S. application Ser. No. 08/799,149, filed Feb. 14, 1997, now U.S. Pat. No. 6,008,195, which claims priority under §119(e)(1) to U.S. Provisional application No. 60/011,834, filed Feb. 16, 1996, the entire contents of which are hereby incorporated by reference herein.

This invention was made with Government support under Grant No. AI22931 awarded by the National Institutes of Health. The Government has certain rights in this invention.

### 1. Field of the Invention

This invention relates generally to antimicrobial peptides, and, more specifically, to peptides designated bovine granulocyte peptide -A (BGP-A), bovine granulocyte peptide -A-amide (BGP-A-amide), murine granulocyte peptide -A (MGP-A) and murine granulocyte peptide -A-amide (MGP-A-amide) and methods of uses thereof.

### 2. Background of the Invention

The cytoplasmic granules of polymorphonuclear leukocytes (neutrophils, granulocytes, PMNs) contain antimicrobial peptides that allow these cells to inactivate ingested microbial targets by mechanisms considered "oxygen independent" (Lehrer, R. I. et al., *Blood* 76:2169-2181, 1990). These granule proteins constitute an antimicrobial arsenal that includes defensins (Selsted, M. E., et al., *Trends in Cell Biology* 5:114-119, 1995),  $\beta$ -defensins (Selsted, M. E., et al., *J. Biol. Chem.* 268:6641-6648, 1993), indolicidin (Selsted, M. E., et al., *J. Biol. Chem.* 267:4292-4295, 1992), and other broad spectrum antibiotic peptides that are released into the phagosome during phagolysosome fusion. To date, members of the defensin family have been isolated from neutrophils of human (Ganz, T., et al., *J. Clin. Invest.* 76:1427-1435, 1985), rabbit (Selsted, M. E., et al., *J. Biol. Chem.* 260:4579-4584, 1985), rat (Eisenhauer, P., et al., *Immun.* 58:3899-3902, 1990), and guinea pig origin (Selsted, M. E., et al., *Infect. Immun.* 55:2281-2286, 1987), and most recently from the Paneth cells of mouse small intestine (Selsted, M. E., et al., *J. Cell Biol.* 118:929-936, 1992).  $\beta$ -defensins have been isolated from the large granules of bovine neutrophils (Selsted, M. E., et al., *J. Biol. Chem.* 268:6641-6648, 1993), bovine tracheal epithelium (Diamond, G. M., et al., *Proc. Natl. Acad. Sci. USA* 88:3952-3956, 1991), and human plasma (Bensch, K. W., et al., *FEBS Lett.* 368:331-335), and indolicidin is a component of the large granules of bovine PMN (Van Abel, R. J., et al., *Int. J. Peptide Protein* 45:401-409, 1995).

The unique features of ruminant granulocytes were first described by Gennaro and Baggiolini and coworkers (Baggiolini, M., et al., *Lab. Invest.* 52:151-158, 1985; Gennaro, R., et al., *J. Cell Biol.* 96:1651-1661, 1983) who demonstrated that neutrophils of cattle, goats, sheep, and ibex are endowed with many unusually large cytoplasmic granules that are distinct from the classical azurophil and specific granules. Subsequent studies established that most of the antibacterial peptides of bovine neutrophils are contained in these unique organelles. Romeo and Gennaro have demonstrated that the large granules of bovine neutrophils contain potent microbicidal peptides that are structurally distinct from defensins (Gennaro, R., et al., *Infect. Immun.* 57:3142-3146, 1989; Romeo, D., et al., *J. Biol. Chem.* 263:9573-9575, 1988). These include three arginine-rich peptides, termed bactenecins, which efficiently kill several gram positive and gram negative bacteria in vitro. Recently, the isolation and characterization of a novel tridecapeptide

2

amide, indolicidin, from bovine neutrophils was reported (Selsted, M. E., et al., *J. Biol. Chem.* 267:4292-4295, 1992). This cationic peptide was shown to be unusually rich in tryptophan, and to have potent bactericidal activity against *E. coli* and *S. aureus*. More recently the isolation of 13  $\beta$ -defensins from bovine neutrophils demonstrated that these peptides are covalently dissimilar to defensins, while possessing a similar folded conformation (Selsted, M. E., et al., *J. Biol. Chem.* 268:6641-6648, 1993).

## SUMMARY OF THE INVENTION

The present invention provides peptides useful as antimicrobial agents. The invention arose from the discovery of a novel tridecapeptide from bovine peripheral blood granulocytes. The purified peptides and their carboxamide analogs have potent antibacterial, antiviral, antiprotozoal, and antifungal activities. These peptides, designated BGP-A and MGP-A, are effective compounds for use in human and/or veterinary medicine, or as agents in agricultural, food science, or industrial applications for example.

The details of the preferred embodiment of the present invention are set forth in the accompanying drawings and the description below. Once the details of the invention are known, numerous additional innovations and changes will become obvious to one skilled in the art.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A and 1B show chromatographs of the purification of BGP-A. FIG. 1A shows the gel filtration chromatography of bovine neutrophil granule extract. FIG. 1B shows the reversed phase HPLC of the peak E fractions.

FIG. 2 shows the analysis of purified BGP-A. FIG. 2A shows the analytical RP-HPLC.

FIG. 2B shows the acid-urea gel of purified BGP-A.

FIG. 3 shows the acid-urea PAGE of purified BGP-A and BGP-A-amide.

FIG. 4 shows the cDNA nucleotide sequence (SEQ ID NO: 2) and the deduced precursor amino acid peptide sequence (SEQ ID NO: 3) of BGP-A.

FIG. 5 shows the cDNA nucleotide sequence (SEQ ID NO: 4) and the deduced precursor amino acid peptide sequence (SEQ ID NO: 5) of MGP-A.

FIG. 6 shows the mature BGP-A (SEQ ID NO: 6) and MGP-A (SEQ ID NO: 7) amino acid sequences. Hatched area indicates identical amino acids conserved between BGP-A and MGP-A. The consensus peptide amino acid sequence is identified as SEQ ID NO: 1.

FIGS. 7A, 7B, 7C and 7D show the microbicidal activities of natural and synthetic BGP-A and synthetic BGP-A-amide.

## DETAILED DESCRIPTION OF THE INVENTION

Before the present nucleic and amino acid sequences, compositions, reagents and methods and uses thereof are described, it is to be understood that this invention is not limited to the particular compositions, reagents, sequences and methodologies described herein as such compositions, reagents, sequences and methodologies may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and that the terminology used herein is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the," include plural referents unless the context clearly indicates otherwise. Thus, for example, reference to "a reagent" includes one or more of such different reagents, reference to "an antibody" includes one or more of such different antibodies, and reference to "the method" includes reference to equivalent steps and methods known to those of ordinary skill in the art that could be modified or substituted for the methods described herein.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention applies. Although any methods, compositions, reagents, sequences similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods and materials are described herein. All publications mentioned herein are incorporated herein, including all figures, graphs, equations, illustrations, and drawings, to describe and disclose specific information for which the reference was cited in connection with.

The publications discussed above are provided solely for their disclosure before the filing date of the present application. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention. Throughout this description, the preferred embodiment and examples shown should be considered as exemplars, rather than as limitations on the present invention.

During the purification of bovine granulocyte  $\beta$ -defensins, antimicrobial activity associated with a small peptide was detected that was different from any previously characterized. Presented herein is the purification, sequencing, synthesis, cDNA isolation, and antimicrobial properties of BGP-A, a thirteen-residue peptide antibiotic expressed in bovine granulocytes. The cDNA for a mouse homolog of BGP-A, isolated from mouse bone marrow and designated MGP-A, is also presented. The deduced MGP-A precursor was remarkably similar to that of BGP-A. The present invention also teaches the synthesis and antimicrobial properties of BGP-A-amide and MGP-A-amide which are analogs of BGP-A and MGP-A respectively.

The invention provides peptide molecules, designated bovine granulocyte peptide -A (BGP-A) and mouse granulocyte peptide -A (MGP-A) and their synthetic carboxamides, designated BGP-A-amide and MGP-A-amide; which exhibit a broad range of antimicrobial and antiprotozoal activity and consequently, are effective antimicrobial agents. Polynucleotides encoding BGP-A and MGP-A represent a new class of antimicrobial peptide genes. As demonstrated by the high conservation of the precursor structure in a ruminant and a rodent, this gene family appears to be remarkably conserved. In a manner similar to the generating of indolicidin (Selsted, M. E., et al., *Peptides: Chemistry and Biology*, ESCOM J. A. Smith and J. E. Rivier, 1992, pp. 905-907), the peptide is synthesized as a much larger prepropeptide and subsequently packaged in granules as the mature product of proteolytic processing. The methods used for the isolation and purification of BGP-A and MGP-A peptides are similar to those previously used for defensin-like peptides; such methods are taught in U.S. Pat. Nos. 4,453,252, 4,659,692, 4,705,777 and 5,242,902, all of which are incorporated by reference herein in their entirety.

As used herein, the term "antimicrobial activity" refers to the ability of a compound to inhibit or irreversibly prevent

the growth of a microorganism. Such inhibition or prevention can be through a microbicidal action or microbistatic inhibition. Therefore, the term "microbicidal inhibition" or "inhibition of microbial growth" as used herein refers to the ability of the antimicrobial peptide to kill, or irrevocably damage the target organism. The term "microbistatic inhibition" as used herein refers to the growth of the target organism without death. Microbicidal or microbistatic inhibition can be applied to an environment either presently exhibiting microbial growth (i.e., therapeutic treatment) or an environment at risk of sustaining or supporting such growth (i.e., prevention or prophylaxis).

As used herein, the term "environment capable of sustaining or supporting microbial growth" refers to a fluid, tissue, space, organ, surface substance or organism where microbial growth can occur or where microbes can exist. Such environments can be, for example, animal tissue; skin or bodily fluids, water and other liquids, food, food products or food extracts, surfaces, crops and certain inanimate objects. It is not necessary that the environment promote the growth of the microbe, only that it permits its subsistence.

The antimicrobial, or antibacterial, activity of BGP-A or MGP-A can be measured against various pathogens by one of ordinary skill in the art. Microorganisms are grown to appropriate concentration, mixed with an appropriate medium, such as an agarose trypticase soy medium, and contacted with BGP-A or MGP-A. After appropriate incubation, the antimicrobial activity is apparent from clear zones surrounding the antibacterial samples. The clear zones are dependent upon the concentration of the peptide. Further methods of determination of antimicrobial activity are taught in Example 5 and in the section entitled "Materials and Methods" herein and are commonly known by those in the art.

Additionally, the minimum inhibitory concentrations (MIC) of BGP-A or MGP-A to effect antimicrobial activity can be determined for a number of different microorganisms according to standard techniques. Briefly, cells are grown overnight at about 37° C. in appropriate bacterial media and diluted in the same medium to give concentrations of about  $10^4$  to  $10^5$  CFU/ml. The broth dilutions are set up in a 96 well microtiter plate, for example, mixing combinations of serially diluted microbes and peptides. After additions of serially diluted bacteria, or other microbes with serially diluted peptide concentrations, the plates are incubated overnight at about 37° C. The next day the plates are scored for the presence or absence of microbial growth in the wells, and the MIC is determined from the scoring.

As used herein, the term's BGP-A, BGP-A-amide, MGP-A and MGP-A-amide refer to peptides or peptidomimetics having generally about 8 to 20 amino acids which make up a chain having a net positive charge. In other words, these are cationic peptides. The peptides of the invention preferably have one or more aromatic amino acids. Illustrative peptide sequences are provided in FIGS. 4-6 and as set forth in SEQ ID NOs: 1, 3, 5, 6 and 7.

The full length BGP-A cDNA is 688 nucleotides in length (SEQ ID NO: 2) with a predicted 21 kD precursor protein composed of 190 residues (SEQ ID NO: 3). Within the precursor peptide, 11 of the first 21 residues are hydrophobic and predict a signal peptide. The signal peptide domain is followed by an intervening propeptide region containing 156 residues. The final 13 residues of the precursor correspond to the mature BGP-A peptide sequence, YKIIQQW-PHYRRV (SEQ ID NO: 6).

The full length MGP-A cDNA is 679 nucleotides in length (SEQ ID NO: 4) and predicts a precursor peptide (SEQ ID

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NO: 5) comprising signal pro-peptide domains similar to those described for BGP-A (FIG. 5). The mature peptide sequence predicted by the murine MGP-A cDNA is identical to BGP-A at 7 of 13 residues (YQVIQSWEHYRE) (FIG. 6; SEQ ID NO: 7). A consensus sequence between the mature BGP and MGP peptides is set forth in FIG. 6 where the hatched area indicates identical amino acids that are conserved between BGP-A and MGP-A and in SEQ ID NO: 1 having an amino acid sequence of YXXIQXWXHYR, where X can be any amino acid. The peptides of the present invention include the SEQ ID NO: 1 consensus sequence. While not wanting to be bound by a particular theory, it is believed that the C-terminus should contain a net positive charge so that the molecule remains active. For example, SEQ ID NO: 1, 6 and 7 all end with an arginine (R) residue, SEQ ID NO: 6 ends with an arginine (R) and valine (V), and SEQ ID NO: 7 ends with a glutamic acid (E) residue. Given that the invention provides both the consensus sequence between mouse and bovine species and the individual DNA sequences encoding the peptides of the present invention, it would not require undue experimentation by the ordinary artisan to isolate homologous BGP/MGP sequences from other species, including human, porcine, ovine, etc., using the teachings supplied herein and methods common in the art (see Maniatis, et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., current edition, incorporated herein by reference).

It should be appreciated that various modifications can be made to the BGP-A or MGP-A amino acid sequences without diminishing the antimicrobial activity of the peptides. It is intended that peptides or peptidomimetics of BGP-A or MGP-A exhibiting such modifications, including amino acid additions, deletions or substitutions are within the scope of the invention. As used herein, the term "substantially the same sequence" refers to a peptide sequence either identical to, or having considerable homology with, for example, the sequences BGP-A or MGP-A as shown in FIGS. 4, 5, and 6 and in SEQ ID NOs: 1, 3, 5, 6 and 7. It is understood that limited modifications can be made to the peptide which result in enhanced function. Likewise, it is also understood that limited modifications can be made without destroying the biological function of the peptide and that only part of the entire primary structure may be required to affect activity. For example, minor modifications of these sequences that do not completely destroy the activity also fall within this definition and within the definition of the compound claimed as such. Modifications can include, for example, additions, deletions, or substitutions of amino acid residues, substitutions with compounds that mimic amino acid structure or function as well as the addition of chemical moieties such as amino and acetyl groups. The modifications can be deliberate or can be accidental such as through mutation in hosts that produce BGP-A or MGP-A peptides exhibiting antimicrobial activity. All these modifications are included as long as the peptide retains its antimicrobial activity.

In some cases, it may be desirable to incorporate one or more non-natural amino acids in the synthetic peptides of the present invention. Possible non-natural amino acids will usually have at least an N-terminus and a C-terminus and will have side chains that are either identical to or chemically modified or substituted from a natural amino acid counter part. An example of a non-natural amino acid is an optical isomer of a naturally-occurring L-amino acid. All peptides were synthesized using L amino acids, however, all D forms of the peptides can be synthetically produced. In

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addition, C-terminal derivatives can be produced, such as C-terminal methyl esters, to increase the antimicrobial activity of a peptide of the invention. Numerous modifications are contemplated according to this invention. Besides the obvious approach of replacement of specific residues in the natural sequence, an alternative embodiment involves synthesis of the peptide from D-amino acids thus reducing potential inactivation by proteases. Such means are well known in the art. (See, for example, Wade et al., *PNAS, USA* 87:4761-4765, 1990.) Examples of chemical modification or substitutions may include hydroxylation or fluorination of C—H bonds within natural amino acids. Such techniques are used in the manufacture of drug analogs of biological compounds and are known to those of ordinary skill in the art. In a preferred embodiment the modification of the peptides of the invention comprises modification by a carboxy terminal amide. Those of skill in the art can make similar substitutions to achieve peptides with greater antimicrobial activity and a broader host range. For example, the invention includes the peptides as set forth in SEQ ID NO:1, 3, 5, 6 and 7, as well as analogues, derivatives or functional fragments thereof, as long as the antimicrobial activity of the peptide remains. Minor modifications of the primary amino acid sequence of the peptides of the invention may result in peptides which have substantially equivalent antimicrobial activity as compared to the specific peptides as set forth in the SEQ ID NOs: 1, 3, 5, 6 and 7 described herein. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the peptides produced by these modifications are included herein as long as the antimicrobial biological activity of the original peptide still exists. BGP-A or MGP-A peptides of the present invention also include functional fragments of the peptide or functional fragments of the nucleic acid sequence encoding the peptide, as long as the activity of BGP-A or MGP-A remains. Smaller peptides containing the biological activity of BGP-A or MGP-A are also included in the invention as are smaller nucleic acid sequences encoding for all or a functional fragment of the peptide. The relative effectiveness of the functional fragments of the peptide or nucleic acid sequences encoding for functional fragments of the peptides of the invention can be readily determined by one of skill in the art by establishing the sensitivity of a microorganism to the peptide fragment. The effectiveness of the peptide functional fragments is assessed by measuring the potential microbicidal or microbistatic activity of the fragment or nucleic acid sequence encoding such a fragment as measured relative to the microbicidal ability of the BGP-A or MGP-A peptides of SEQ ID NO: 6 or 7 respectively. Testing is carried out as described in the section titled "Antimicrobial Assay" in the Materials and Methods section herein and in Example 5 of the present invention or by other standard antimicrobial tests (e.g., MIC) commonly known to those in the art.

Further, deletion of one or more amino acids can also result in a modification of the structure of the resultant peptide without significantly altering its biological activity. This can lead to the development of a smaller active peptide which would also have utility. For example, amino or carboxy terminal amino acids which may not be required for biological activity of the particular peptide can be removed. Peptides of the invention include any analog, homolog, mutant, isomer or derivative of the peptides disclosed in the present invention, so long as the bioactivity as described herein is remains. The methods and compositions of the present invention may also employ synthetic non-peptide compositions that have biological activity functionally com-

parable to that of BGP-A, MGP-A, BGP-A-Amide, or MGP-A-Amide. By "functionally comparable," it is meant that the shape, size, flexibility, and electronic configuration of the non-peptide molecule are such that the biological activity of the molecule is similar to the BGP-A, MGP-A, BGP-A-Amide, or MGP-A-Amide peptides. In particular, the non-peptide molecules should display comparable antimicrobial activity. Such non peptide molecules can be small molecules having a molecular weight in the range of about 100 to 1000 Daltons. The use of such small molecules is advantageous in the preparation of pharmacological compositions.

The identification of such non-peptide analog molecules can be performed using techniques known in the art of drug design. Such techniques include, but are not limited to, self-consistent field (SCF) analysis, configuration interaction (CI) analysis, and normal mode dynamics computer analysis, all of which are well described in the scientific literature. See, e.g., Rein et al., *Computer-Assisted Modeling of Receptor-Ligand Interactions*, Alan Liss, N.Y., (1989). Preparation of the identified compounds will depend on the desired characteristics of the compounds will involve standard chemical synthetic techniques. See, Cary et al., *Advanced Organic Chemistry*, part B, Plenum Press, New York (1983).

The term "conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine, and the like. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted peptide also immunoreact with the unsubstituted peptide.

The BGP-A or MGP-A peptides of the present invention can be synthesized by methods well known in the art, such as through the use of automatic peptide synthesizers, by recombinant methods or well-known manual methods of peptide synthesis. In addition, they can be purified from natural sources such as white blood cells and from bone marrow of a vertebrate, preferably of mammalian origin. Such cells or tissues can be obtained by means well known to those skilled in the art.

The term "substantially pure" as used herein refers to BGP-A or MGP-A nucleic acid or protein which is substantially free of other proteins, lipids, carbohydrates or other materials with which it is naturally associated or that the peptide or protein so designated has been separated from its in vivo cellular environment. Because of the separation and purification, the substantially pure peptides and proteins are useful in ways that the non-separated impure peptides or proteins are not. One skilled in the art can purify BGP-A or MGP-A using standard techniques for protein purification. The substantially pure peptide will yield a single major band on an acid-urea gel. The purity of the BGP-A or MGP-A peptide can also be determined by amino-terminal amino acid sequence analysis and analytical RP-HPLC.

The invention also provides polynucleotides encoding the BGP-A or MGP-A protein. These polynucleotides include DNA, cDNA and RNA sequences which encode BGP-A or MGP-A. It is understood that all polynucleotides encoding all or a portion of BGP-A or MGP-A are also included herein, as long as they encode a peptide with BGP-A or

MGP-A activity. Such polynucleotides include naturally occurring, synthetic, and intentionally manipulated polynucleotides. For example, BGP-A or MGP-A polynucleotide may be subjected to site-directed mutagenesis. The polynucleotides of the invention include sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the invention as long as the amino acid sequence of BGP-A or MGP-A peptide encoded by the nucleotide sequence is functionally unchanged. The polynucleotide encoding BGP-A or MGP-A includes the nucleotide sequence in FIGS. 4 and 5 (SEQ ID NOs: 2 and 4), as well as complementary nucleic acid sequences. A complementary sequence may include an antisense nucleotide. When the sequence is RNA, the deoxynucleotides A, G, C, and T of SEQ ID Nos: 2 and 4 are replaced by ribonucleotides A, G, C, and U, respectively. Also included in the invention are fragments of the above-described nucleic acid sequences that are at least 15 bases in length, which is sufficient to permit the fragment to selectively hybridize to DNA (SEQ ID NOs: 2 and 4) that encodes the protein of FIGS. 4 and 5 (SEQ ID NOs: 3 and 5), under physiological conditions.

Also, provided by this invention are the nucleic acid sequences encoding the BGP-A or MGP-A peptides, vectors and host cells containing them and methods of expression to provide recombinantly produced peptides. This method comprises growing the host cell containing a nucleic acid encoding a peptide under suitable conditions such that the nucleic acid is transmitted and/or translated and isolating the peptide so produced.

After the peptide of this invention is isolated, nucleic acids encoding the peptides are isolated by methods well known in the art, *infra*. These isolated nucleic acids can be ligated into vectors and introduced into suitable host cells for expression. Methods of ligation and expression of nucleic acids within cells are well known in the art, (see Maniatis, et al., *Molecular Cloning. A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., current edition, incorporated herein by reference).

Specifically disclosed herein is a cDNA sequence containing the active portion of the BGP-A or MGP-A coding sequence. One of skill in the art could now use this sequence to isolate other full length clones. The full length BGP-A cDNA is 688 nucleotides in length (SEQ ID NO: 2) and predicts a 21 kD precursor composed of 190 residues (FIG. 4; SEQ ID NO: 3). Within the BGP-A precursor, 11 of the first 21 residues are hydrophobic and predict a signal peptide (Von Heijne, G., *Eur. J. Biochem.* 133:17-21, 1983). The signal peptide domain is followed by an intervening propeptide region containing 156 residues. The final 13 residues of the precursor correspond to the mature BGP-A peptide sequence (SEQ ID NO: 6). The full-length MGP-A cDNA is 679 nucleotides in length (SEQ ID NO: 4) and predicts a precursor comprising signal propeptide domains similar to those described for BGP-A (FIG. 5; SEQ ID NO: 5). Based on this similarity, this sequence isolated from murine bone marrow cDNA is designated as murine neutrophil peptide A (MGP-A; FIG. 5; SEQ ID NOs: 5 and 7). The mature peptide sequence predicted by the murine cDNA is identical to BGP-A at 7 of 13 residues (FIG. 6; SEQ ID NO: 7). The hatched area in FIG. 6 indicates identical amino acids conserved between BGP-A and MGP-A. The consensus peptide amino acid sequence is YXXIQXWXHYR (SEQ ID NO: 1), where X can be any amino acid.

DNA sequences of the invention can be obtained by several methods. For example, the DNA can be isolated

using hybridization techniques which are well known in the art. These include, but are not limited to: 1) hybridization of genomic or cDNA libraries with probes to detect homologous nucleotide sequences, 2) polymerase chain reaction (PCR) on genomic DNA or cDNA using primers capable of annealing to the DNA sequence of interest, and 3) antibody screening of expression libraries to detect cloned DNA fragments with shared structural features. The sequences of a pair of nucleic acid molecules (or two regions within a single nucleic acid molecule) are said to be "complementary" to each other if base pairing interactions can occur between each nucleotide of one of the members of the pair and each nucleotide of the other member of the pair. A pair of nucleic acid molecules (or two regions within a single nucleic acid molecule) are said to "hybridize" to each other if they form a duplex by base pairing interactions between them. As known in the art, hybridization between nucleic acid pairs does not require complete complementarity between the hybridizing regions, but only that there is a sufficient level of base pairing to maintain the duplex under the hybridization conditions used.

Hybridization reactions are typically carried out under low to moderate stringency conditions, in which specific and some nonspecific interactions can occur. After hybridization, washing can be carried out under moderate or high stringency conditions to eliminate nonspecific binding. As known in the art, optimal washing conditions can be determined empirically, e.g., by gradually increasing the stringency. Condition parameters that can be changed to affect stringency include, e.g., temperature and salt concentration. In general, the lower the salt concentration and the higher the temperature, the higher the stringency. For example, washing can be initiated at a low temperature (e.g., room temperature) using a solution containing an equivalent or lower salt concentration as the hybridization solution. Subsequent washing can be carried out using progressively warmer solutions having the same salt solution. Alternatively, the salt concentration can be lowered and the temperature maintained in the washing step, or the salt concentration can be lowered and the temperature increased. Additional parameters can be altered to affect stringency, including, e.g., the use of a destabilizing agent, such as formamide.

In nucleic acid hybridization reactions, the conditions used to achieve a particular level of stringency will vary, depending on the nature of the nucleic acids being hybridized. For example, the length, degree of complementarity, nucleotide sequence composition (e.g., GC v. AT content), and nucleic acid type (e.g., RNA v. DNA) of the hybridizing regions of the nucleic acids can be considered in selecting hybridization conditions. An additional consideration is whether one of the nucleic acids is immobilized, for example, on a filter.

An example of progressively higher stringency conditions is as follows: 2xSSC/0.1% SDS at about room temperature (hybridization conditions); 0.2xSSC/0.1% SDS at about room temperature (low stringency conditions); 0.2xSSC/0.1% SDS at about 42° C. (moderate stringency conditions); and 0.1xSSC at about 68° C. (high stringency conditions). Washing can be carried out using only one of these conditions, e.g., high stringency conditions, or each of the conditions can be used, e.g., for 10–15 minutes each, in the order listed above, repeating any or all of the steps listed. However, as mentioned above, optimal conditions will vary, depending on the particular hybridization reaction involved, and can be determined empirically.

Preferably the BGP-A or MGP-A polynucleotide of the invention is derived from a mammalian organism, and most

preferably from a mouse, cow, or human. Screening procedures which rely on nucleic acid hybridization make it possible to isolate any gene sequence from any organism, provided the appropriate probe is available. Oligonucleotide probes, which correspond to a part of the sequence encoding the protein in question, can be synthesized chemically. This requires that short, oligopeptide stretches of amino acid sequence must be known. The DNA sequence encoding the protein can be deduced from the genetic code, however, the degeneracy of the code must be taken into account. It is possible to perform a mixed addition reaction when the sequence is degenerate. This includes a heterogeneous mixture of denatured double stranded DNA. For such screening, hybridization is preferably performed on either single-stranded DNA or denatured double-stranded DNA. Hybridization is particularly useful in the detection of cDNA clones derived from sources where an extremely low amount of mRNA sequence relating to the peptide of interest is present. In other words, by using stringent hybridization conditions directed to avoid nonspecific binding, it is possible, for example, to allow the autoradiographic visualization of a specific cDNA clone by the hybridization of the target DNA to that single probe in the mixture which is its complete complement (Wallace, et al., *Nucl. Acid Res.*, 9:879,1981).

Therefore, given a partial DNA sequence of the BGP-A or MGP-A gene of interest, one of skill in the art would be able to prepare probes for isolation of a full length cDNA clone, without undue experimentation (see for example, Ausubel, et al., *Current Protocols in Molecular Biology*, Units 6.3–6.4, Greene Publ., 1994; Maniatis, et al., *Molecular Cloning*, Cold Spring Harbor Laboratories, current edition).

The complement of specific DNA sequences encoding BGP-A or MGP-A can also be obtained by: 1) isolation of double-stranded DNA sequences from the genomic DNA; 2) chemical manufacture of a DNA sequence to provide the necessary codons for the peptide of interest; and 3) in vitro synthesis of a double-stranded DNA sequence by reverse transcription of mRNA isolated from a eukaryotic donor cell. In the latter case, a double-stranded DNA complement of mRNA is eventually formed which is generally referred to as cDNA. Of the three above-noted methods for developing specific DNA sequences for use in recombinant procedures, the isolation of genomic DNA isolates is the least common. This is especially true when it is desirable to obtain the microbial expression of mammalian peptides due to the presence of introns.

The synthesis of DNA sequences is frequently the method of choice when the entire sequence of amino acid residues of the desired peptide product is known. When the entire sequence of amino acid residues of the desired peptide is not known, the direct synthesis of DNA sequences is not possible and the method of choice is the synthesis of cDNA sequences. Among the standard procedures for isolating cDNA sequences of interest is the formation of plasmid- or phage-carrying cDNA libraries which are derived from reverse transcription of mRNA which is abundant in donor cells that have a high level of genetic expression. When used in combination with polymerase chain reaction technology, even rare expression products can be cloned. In those cases where significant portions of the amino acid sequence of the peptide are known, the production of labeled single or double-stranded DNA or RNA probe sequences duplicating a sequence putatively present in the target cDNA may be employed in DNA/DNA hybridization procedures which are carried out on cloned copies of the cDNA which have been denatured into a single-stranded form (Jay, et al., *Nucl. Acid*, 11:2325, 1983).

Several types of vectors are available and can be used to practice this invention, e.g., plasmid, DNA and RNA viral vectors, baculoviral vectors, and vectors for use in yeast. When the vector is a plasmid, it generally contains a variety of components including promoters, signal sequences, phenotypic selection genes, origins of replication sites, and other necessary components as are known to those of skill in the art.

Promoters most commonly used in prokaryotic vectors include the lac Z promoter system, the alkaline phosphatase pho A promoter, the bacteriophage XPL promoter (a temperature sensitive promoter), the tac promoter (a hybrid trp-lac promoter regulated by the lag repressor), the tryptophan promoter, and the bacteriophage T7 promoter.

One other useful component of vectors used to practice this invention is a signal sequence. This sequence is typically found immediately 5' to the nucleic acid encoding the peptide, and will thus be transcribed at the amino terminus of the fusion protein. However, in certain cases, the signal sequence has been demonstrated to be at positions other than 5' to the gene encoding the protein to be secreted. This sequence targets the protein to which it is attached across the inner membrane of the bacterial cell. The DNA encoding the signal sequence can be obtained as a restriction endonuclease fragment from any nucleic acid encoding a peptide that has a signal sequence. Suitable prokaryotic signal sequences can be obtained from genes encoding, for example Lamb or OmpF (Wong, et al, *Gene* 68:193, 1983), MalE, PhoA, OmpA and other genes. A preferred prokaryotic signal sequence for practicing this invention is the *E. coli* heatstable enterotoxin II (STII) signal sequence as described by Chang, et al, *Gene* 55:189, 1987.

Another useful component of the vectors used to practice this invention is a phenotypic selection gene. Typical phenotypic selection genes are those encoding proteins that confer antibiotic resistance upon the host cell. By way of illustration, the ampicillin resistance gene (amp) and the tetracycline resistance gene (tet) are readily employed for this purpose.

Construction of suitable vectors comprising the aforementioned components as well as the gene encoding the desired peptide are prepared using standard recombinant DNA procedures. Isolated DNA fragments to be combined to form the vector are cleaved, tailored, and ligated together in a specific order and orientation to generate the desired vector.

The DNA is prepared according to standard procedures (see Maniatis, et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., current edition, incorporated herein by reference). If the DNA fragment is to be ligated into a vector, the vector is at first linearized by cutting with the appropriate restriction endonucleases. The linearized vector can then be treated with alkaline phosphatase or calf intestinal phosphatase. The phosphatasing prevents self-ligation of the vector during the ligation step.

After ligation, the vector with the heterologous gene now inserted is transformed into a suitable host cell. Suitable prokaryotic host cells include *E. coli* strain JM101, *E. coli* K12 strain 294 (ATCC number 31,446), *E. coli* strain W3110 (ATCC number 27,325), *E. coli* X1776 (C number 31, 537), *E. coli* XL-1Blue (Stratagene), and *E. coli* B; however, many other strains of *E. coli*, such as HB101, NM522, NM538, NM539 and many other species and genera of prokaryotes can be used as well. Besides the *E. coli* strains listed above, bacilli such as *Bacillus subtilis*, other enterobacteriaceae

such as *Salmonella typhimurium* or *Serratia marcesans* and various *Pseudomonas* species can all be used as hosts.

Transformation of prokaryotic cells is readily accomplished using calcium chloride or other methods well known to those skilled in the art. Electroporation (Neumann, et al., *EMBO J.* 1:841 1982) also can be used to transform these cells. The transformed cells are selected by growth on an antibiotic, commonly tetracycline (tet) or ampicillin (amp), to which they are rendered resistant due to the presence of tet and/or amp resistance genes on the vector.

After selection of the transformed cells, these cells are grown in culture and the plasmid DNA (or other vector with the foreign gene inserted) is then isolated. Plasmid DNA can be isolated using methods known in the art. This purified plasmid DNA is then analyzed by restriction mapping and/or DNA sequencing.

Following procedures outlined above, mammalian cell lines such as myeloma (P3-653), hybridoma (SP2/0), Chinese Hamster Ovary (CHO), Green monkey kidney (COSI) and murine fibroblasts (L492) are suitable host cells for peptide expression. These "mammalian" vectors can include a promoter, an enhancer, a polyadenylation signal, signal sequences and genes encoding selectable markers such as geneticin (neomycin resistance), mycophenolic acid (xanthine guanine phosphoribosyl transferase) or histidinol (histidinol dehydrogenase). Suitable promoters for use in mammalian host cells include, but are not limited to, Ig Kappa, Ig Gamma, Cytomegalovirus (CMV) immediate early, Rous Sarcoma Virus (RSV), Simian virus 40 (SV40) early, mouse mammary tumor (MMTV) virus and metallothionein. Suitable enhancers include, but are not limited to, Ig Kappa, Ig Heavy, CMV early and SV40. Suitable polyadenylation sequences include Ig Kappa, Ig Gamma or SV40 large T antigen. Suitable signal sequences include Ig Kappa, Ig Heavy and human growth hormone (HGH).

When the vector is baculovirus, suitable promoters and enhancer sequences include, but are not limited to, AcMGPV polyhedrin, AcMGPV ETL and AcMGPV p10 sequences. One particularly suitable polyadenylation signal is the polyhedrin AcMGPV. Ig Kappa, Ig Heavy and AcMGPV are examples of suitable signal sequences. These vectors are useful in the following insect cell lines, among others: SF9, SF21 and High 5.

Alternatively, the peptides can be expressed in yeast strains such as PS23-6A, W301-18A, LL20, D234-3, INVSC1, INVSC2, YJJ337. Promoter and enhancer sequences such as gal 1 and pEFT-1 are useful. Vra-4 also provides a suitable enhancer sequence. Sequences useful as functional "origins of replication" include ars1 and 2μ circular plasmid.

The invention includes antibodies that are immunoreactive with BGP-A or MGP-A peptides or fragments thereof. Antibodies which consist essentially of pooled monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibody preparations are provided. Monoclonal antibodies are made from antigen containing fragments of the protein by methods well known to those skilled in the art (Kohler, et al., *Nature* 256:495, 1975). Anti-BGP-A or MGP-A antibodies can be made by methods conventional in the art. For example, polyclonal antiserum can be raised in appropriate animals, such as rabbits, mice, or rats. BGP-A or MGP-A peptides, either synthetically obtained or naturally obtained, can be used to immunize the animal. The immunogen can then be used to immunize animals by means well known to those skilled in the art. Serum samples are collected until the anti-BGP-A or

MGP-A titer is appropriate. Various fractions of the antisera, such as IgG, can be isolated by means well known in the art. Alternatively, BGP-A or MGP-A immunogens can be used to obtain monoclonal antibodies, again by means well known in the art. (See, for example, Harlow et al., *Antibodies: A Laboratory Manual*, Cold Springs Harbor Laboratory, 1988.)

Anti-BGP-A or MGP-A antibodies can be used to detect the presence of BGP-A or MGP-A in biological samples, such as histological samples. An appropriate detectable second antibody can be used to identify the primary antibody attached to the BGP-A or MGP-A by visualization. Means of detection include the use of radioactive nucleotides or enzyme substrates such as peroxidase. For example, anti-BGP-A was produced by standard methods and shown to stain bone marrow preparations from cattle (cytological sample). In particular, granulocytes (e.g., eosinophils) were stained heavily for BNP-A.

The term "antibody" as used in this invention includes intact molecules as well as fragments thereof, such as Fab, Fab', F(ab')<sub>2</sub>, and Fv that can bind the epitopic determinant. These antibody fragments retain some ability selectively to bind with its antigen or receptor and are defined as follows:

- (1) Fab, the fragment that contains a monovalent antigen-binding fragment of an antibody molecule can be produced by digestion of whole antibody with the enzyme pepsin to yield an intact light chain and part of one heavy chain;
- (2) Fab', the fragment of an antibody molecule can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and part of the heavy chain; two Fab' fragments are obtained per antibody molecule;
- (3) (Fab')<sub>2</sub>, the fragment of the antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; F(ab')<sub>2</sub> is a dimer of two Fab' fragments held together by two disulfide bonds;
- (4) Fv, defined as a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain expressed as two chains; and
- (5) Single chain antibody ("SCA"), defined as a genetically engineered molecule containing the variable region of the light chain, the variable region of the heavy chain, linked by a suitable peptide linker as a genetically fused single chain molecule.

Methods of making these fragments are known in the art. (See for example, Harlow and Lane, *Antibodies. A Laboratory Manual*, Cold Spring Harbor Laboratory, N.Y. (current edition), incorporated herein by reference).

As used in this invention, the term "epitope" means any antigenic determinant on an antigen to which the paratope of an antibody binds. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics.

If needed, polyclonal or monoclonal antibodies can be further purified, for example, by binding to and elution from a matrix to which the peptide or a peptide to which the antibodies are raised is bound. Those of skill in the art will know of various techniques common in the immunology arts for purification and/or concentration of polyclonal antibodies, as well as monoclonal antibodies (See, e.g., Coligan, et al., Unit 9, *Current Protocols in Immunology*, Wiley Interscience, current edition, incorporated by reference).

It is also possible to use the anti-idiotypic technology to produce monoclonal antibodies that mimic an epitope. For example, an anti-idiotypic monoclonal antibody made to a first monoclonal antibody will have a binding domain in the hypervariable region that is the "image" of the epitope bound by the first monoclonal antibody.

The phrase "purified antibody" means an antibody that is at least 60%, by weight, free from proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably 90%, and most preferably at least 99%, by weight, an antibody, e.g., an anti-BGP-A specific antibody. A purified antibody may be obtained, for example, by affinity chromatography using recombinantly-produced protein or conserved motif peptides and standard techniques. The invention can employ not only intact monoclonal or polyclonal antibodies, but also an immunologically-active antibody fragment, such as a Fab, Fab' or (Fab')<sub>2</sub> fragments, or a genetically engineered Fv fragment (Ladner et al., U.S. Pat. No. 4,946,788).

"Specifically binds" means an antibody that recognizes and binds a specified protein, e.g., an anti-BGP-A, specific antibody or anti-MGP-A specific antibody, which does not substantially recognize and bind other molecules in a sample which naturally includes protein.

It should be understood that the compositions of the present invention have activity against many microorganisms, such as fungi, bacteria (both gram positive and negative), and protozoa and viruses. Different compositions will have differing degrees of activities toward different organisms. The peptides of the present invention may also be combined with other proteins to act as preservatives to protect the proteins against bacterial degradation. Alternatively, the subject peptides or compositions may be used as preservatives and disinfectants in many formulations, such as contact lens solutions, ointments, shampoos, medicaments, foods, and the like. The amount of peptide employed in the compositions may vary depending upon the nature of the other components, how much protection is required and the intended use of the composition.

In a preferred embodiment, the present invention provides administration of a therapeutic amount of an antimicrobial peptide of the invention. One or more of the peptides disclosed herein, may have utility as antifungal agents, either alone, or as lipid fascicle preparations. The latter approach has been used with success with the non-peptide antifungal drug amphotericin. Specific applications would be dependent on the pathogen targeted. For example, *C. albicans*, the common cause of mucocutaneous fungal disease in AIDS patients, which is extremely susceptible to several  $\beta$ -defensins, might be controlled in these individuals more effectively by a BGP-A or MGP-A based therapeutic or in combination with existing first line drugs. Similarly, BGP-A or MGP-A may be used therapeutically in veterinary medicine. One advantage of the therapeutic use of the present invention is that the peptides exhibit low immunogenicity.

BGP-A or MGP-A, either purified from natural sources or synthetic, can be administered to a subject in need of therapy by various means, including oral administration, preferably in a slow-release type formulation that will avoid release within the stomach. Alternatively, they can be administered through a nasal gastric incubation or transabdominal catheter. Individual species of BGP-A or MGP-A can be administered singly or a combination can be administered simultaneously or sequentially and also with other antimicrobial compositions.

The invention further provides a pharmaceutical composition for treating a human bacterial or fungal infection that comprises the purified peptide of the invention in an amount effective to treat a human bacterial or fungal infection and a pharmaceutically acceptable carrier.

The method of inhibiting the growth of bacteria may further include the addition of antibiotics for combination or synergistic therapy. The appropriate antibiotic administered will typically depend on the susceptibility of the bacteria such as whether the bacteria is gram negative or gram positive, and will be easily discernable by one of skill in the art. Examples of particular classes of antibiotics useful for synergistic therapy with the peptides of the invention include aminoglycosides (e.g., tobramycin), penicillins (e.g., piperacillin), cephalosporins (e.g., ceftazidime), fluoroquinolones (e.g., ciprofloxacin), carbapenems (e.g., imipenem), tetracyclines and macrolides (e.g., erythromycin and clarithromycin). The method of inhibiting the growth of bacteria may further include the addition of antibiotics for combination or synergistic therapy. The appropriate antibiotic administered will typically depend on the susceptibility of the bacteria such as whether the bacteria is gram negative or gram positive, and will be easily discernable by one of skill in the art. Further to the antibiotics listed above, typical antibiotics include aminoglycosides (amikacin, gentamicin, kanamycin, netilmicin, tobramycin, streptomycin, azithromycin, clarithromycin, erythromycin, erythromycin estolate/ethylsuccinate/glucetate/lactobionate/stearate), beta-lactams such as penicillins (e.g., penicillin G, penicillin V, methicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin, ampicillin, amoxicillin, ticarcillin, carbenicillin, mezlocillin, azlocillin and piperacillin), or cephalosporins (e.g., cephalothin, cefazolin, cefaclor, cefamandole, cefoxitin, cefuroxime, cefonicid, cefmetazole, cefotetan, cefprozil, loracarbef, cefetamet, cefoperazone, cefotaxime, ceftizoxime, ceftriaxone, ceftazidime, cefepime, cefixime, cefpodoxime, and cefsulodin). Other classes of antibiotics include carbapenems (e.g., imipenem), monobactams (e.g., aztreonam), quinolones (e.g., fleroxacin, nalidixic acid, norfloxacin, ciprofloxacin, ofloxacin, enoxacin, lomefloxacin and cinoxacin), tetracyclines (e.g., doxycycline, minocycline, tetracycline), and glycopeptides (e.g., vancomycin, teicoplanin), for example. Other antibiotics include chloramphenicol, clindamycin, trimethoprim, sulfamethoxazole, nitrofurantoin, rifampin and mupirocin.

In certain embodiments of the invention, the treatment of the soluble proteins comprises size exclusion chromatography, ion-exchange chromatography, or reverse phase, high performance, liquid chromatography. It will be appreciated by one skilled in the art, however, that treatment of soluble proteins to purify peptides may be accomplished by many methods known to those skilled in the art, all of which are contemplated by this invention. Further, in one embodiment of the invention, the treatment of granulocytes to recover granules comprises density gradient centrifugation.

The invention also provides a composition that comprises the purified peptide in an amount effective to kill bacteria or fungi and a suitable carrier. Such composition may be used in numerous ways to combat bacteria or fungi, for example, in household or laboratory antimicrobial formulations using carriers well known in the art.

The compositions of the present invention can comprise the BGP-A, BGP-A-Amide, MGP-A, or MGP-A-Amide, either singly or in combination, incorporated in a physiologically-acceptable-carrier suitable for topical application. The compositions may contain from about 10 ug/ml

to 2000 ug/ml, preferably 50 ug/ml to 500 ug/ml. The nature of the carrier will vary depending on the intended area of application. For application to the skin, a cream or an ointment base is usually preferred with suitable bases including lanolin, Silvadene™ (Marion; particularly for the treatment of burns) Aquaphor™ (Duke Laboratories, South Norwalk, Conn.), and the like. It will also be possible to incorporate the BGP-A, BGP-A-Amide, MGP-A, or MGP-A-Amide peptides in natural and synthetic bandages and other wound dressings to provide for continuous exposure of a wound to the peptides. Aerosol applicators may also find use with the present invention.

Where the peptides are to be used as antimicrobial agents, they can be formulated in buffered aqueous media containing a variety of salts and buffers. The salts will for the most parts be alkali and alkaline earth halides, phosphates and sulfates, e.g., sodium chloride, potassium chloride or sodium sulfate. Various buffers may be used, such as citrate, phosphate, HEPES, Tris or the like to the extent that such buffers are physiologically acceptable to the host that is being treated.

Various excipients or other additives may be used, where the compounds are formulated as lyophilized powders, for subsequent use in solution. The excipients may include various polyols, inert powders or other extenders.

Depending on the nature of the formulation and the host, the subject compounds may be administered in a variety of ways. The formulations may be applied topically, by injection, e.g., intravenously, intraperitoneal, nasopharyngeal, etc.

In another aspect of the invention, compositions comprising the purified peptide of the invention in a microbicidal effective amount and a suitable carrier or pharmaceutical composition, or pharmaceutically acceptable carrier may additionally comprise a detergent. The addition of a detergent to such peptide compositions is useful to enhance the antibacterial, antiviral, or antifungal characteristics of the novel peptide of the invention. Although any suitable detergent may be used, the presently preferred detergent is a nonionic detergent, such as Tween 20 or 1% NP40.

The invention also provides a pharmaceutical formulation or composition for treating a human microbial, bacterial, viral, or fungal infection that comprises the purified peptide of the invention or a gene delivery and gene expression vector that can deliver an effective amount of peptide in an amount effective to treat a human microbial bacterial, viral, or fungal infection incorporated into a pharmaceutically acceptable liposome or other delivery vehicle.

"Formulation" means a composition capable of gene delivery and gene expression, which can deliver a nucleotide sequence to, or directly into, a target cell whereupon the formulation containing the nucleotide sequence is incorporated on the cytoplasmic side of the outermost membrane of the target cell and capable of achieving gene expression so that detectable levels of gene expression of the delivered nucleotide sequence are expressed in the target cell. More preferably, after delivery into the cytoplasmic side of the cell membrane the composition is subsequently transported, without undergoing endosomal or lytic degradation, into the nucleus of the target cell in a functional state capable of achieving gene expression so that detectable levels of gene expression of the delivered nucleotide sequence are expressed in the target cell. Expression levels of the gene or nucleotide sequence inside the target cell can provide gene expression for a duration of time and in an amount such that the nucleotide product therein can provide a biologically beneficially effective amount of a gene product or in such an

amount as to provide a functionally beneficial biological effect. As used herein, the term formulation can refer to, but is not limited by (either explicitly or implicitly) the following examples: (1) liposome or liposome reagents or liposomal compositions either cationic, anionic or neutral in net character and net charge; (2) DNA, nucleic acid or a nucleic acid expression vector ionically complexed with a polycation/s and a ligand/s such that after attachment of the [DNA+Polycation+Ligand] composition to a cell surface receptor on a target cell via the ligand, the [DNA+Polycation+Ligand] composition can be endocytosed into the target cell and the DNA is subsequently decoupled from the ligand and polycation and delivered to the cell nucleus in a functional condition for subsequent expression. Various alterations in the composition can be envisioned by those of ordinary skill in the art such as including peptide sequences that (a) protect the composition from endosomal lysis after incorporation into the target cell by allowing the composition to leave the lysosomal vesicle, or (b) which act as a nuclear targeting agent, chaperoning the nucleic acid through the pores of the nuclear envelope and into the nucleus of the cell. Similar reagents, which have been previously described, are the asialoglycoprotein-polylysine conjugations (Wu et al., *J. Biol. Chem.* 263:14621, 1988; Wu et al., *J. Biol. Chem.* 264:16985, 1989); (3) naked nucleic acid; (4) compacted nucleic acid or a compacted reagent; or (5) plasmid or naked DNA that can be microinjected (Wolff et al., *Science* 247:1465, 1990); (6) nucleic acid in a viral or retroviral vector composition; and (7) colloidal dispersions (Felgner et al., *Proc. Natl. Acad. Sci. USA* 84:7413, 1987; Ono et al., *Neuroscience Lett.* 117:259, 1990; Brigham et al., *Am. J. Med. Sci.* 298:278, 1989; Staubinger and Papahadjopoulos, *Meth. Enz.* 101:512, 1983). One of ordinary skill in the art will recognize that other compositions for the delivery of nucleotide sequences to target cells may be envisioned.

It will be readily understood by those skilled in the art that any suitable pharmaceutically acceptable liposome may be used as a vehicle for the peptide of the present invention. Such liposomal compositions have activity against many microorganisms similar to the activity of other compositions of this invention discussed in more detail above. Additionally, these compositions may be administered in a variety of conventional and well-known ways as is also discussed in greater detail above.

"Therapeutically effective" as used herein, refers to an amount of formulation, composition, or reagent in a pharmaceutical acceptable carrier that is of sufficient quantity to ameliorate the state of the patient or animal so treated. "Ameliorate" refers to a lessening of the detrimental effect of the disease state or disorder in the recipient of the therapy. The subject of the invention is preferably a human, however, it can be envisioned that any animal can be treated in the method of the instant invention. The term "modulate" means enhance, inhibit, alter, or modify the expression or function of antimicrobial activity in combination with a pharmaceutically acceptable carrier.

Pharmaceutically acceptable carrier preparations for administration include sterile or aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils.

The active therapeutic ingredient is often mixed with excipients that are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients include water, saline, dextrose, glycerol and ethanol, or combinations thereof. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present such as, for example, antimicrobial, antioxidants, chelating agents, and inert gases and the like.

Another therapeutic approach included within the invention involves direct administration of reagents or compositions by any conventional administration techniques (for example but not restricted to local injection, inhalation, or administered systemically), to the subject with a microbial, bacterial, viral or fungal disorder. The reagent, formulation or composition may also be targeted to specific cells or receptors by any of the methods described herein. The actual dosage of reagent, formulation or composition that modulates a microbial, bacterial, viral or fungal disorder depends on many factors, including the size and health of an organism, however one of ordinary skill in the art can use the following teachings describing the methods and techniques for determining clinical dosages (Spilker B., *Guide to Clinical Studies and Developing Protocols*, Raven Press Books, Ltd., New York, 1984, pp. 7-13, 54-60; Spilker B., *Guide to Clinical Trials*, Raven Press, Ltd., New York, 1991, pp. 93-101; Craig C., and R. Stitzel, eds., *Modern Pharmacology*, 2d ed., Little, Brown and Co., Boston, 1986, pp. 127-33; T. Speight, ed., *Avery's Drug Treatment: Principles and Practice of Clinical Pharmacology and Therapeutics*, 3d ed., Williams and Wilkins, Baltimore, 1987, pp. 50-56; R. Tallarida, R. Raffa and P. McGonigle, *Principles in General Pharmacology*, Springer-Verlag, New York, 1988, pp. 18-20) to determine the appropriate dosage to use; but, generally, in the range of about 0.1 mg/kg to 1000 mg/kg, more specifically between about 1.0 mg/kg and 500 mg/kg, and preferably from about 10 mg/kg and 100 mg/kg inclusive final concentration are administered per day to an adult in any pharmaceutically-acceptable carrier.

The peptides of the present invention can also be used to treat an LPS associated disorder. With reference to an LPS associated disorder, the term "therapeutically effective amount" as used herein for treatment of an LPS associated disorder such as endotoxemia or sepsis refers to the amount of BGP-A or MGP-A peptide sufficient to decrease the subject's response to LPS and decrease the symptoms of an LPS associated disorder, such as sepsis. The term "therapeutically effective" therefore includes that the amount of BGP-A or MGP-A peptide sufficient to prevent, and preferably reduce by at least 50%, and more preferably sufficient to reduce by 90%, a clinically significant increase in the plasma level of LPS. The dosage ranges for the administration of BGP-A or MGP-A peptide are those large enough to produce the desired effect. Generally, the dosage will vary with the age, condition, sex, and extent of the infection with bacteria or other agent as described above, in the patient and can be determined by one skilled in the art. The dosage can be adjusted by the individual physician in the event of any contraindications. In any event, the effectiveness of treatment can be determined by monitoring the level of LPS or LPS associated molecules, such as tumor necrosis factor (TNF), in a patient. A decrease in serum LPS and TNF levels correlates positively with amelioration of the LPS associated disorder.

In a further embodiment, the present invention may be used as a food preservative or in treating food products to

eliminate potential pathogens. The latter use might be targeted to the fish and poultry industries that have serious problems with enteric pathogens which cause severe human disease. In another embodiment, BGP-A or MGP-A may be used as disinfectants, for use in any product that must remain microbial free. In a further embodiment, BGP-A or MGP-A may be used as antimicrobials for food crops, either as agents to reduce post harvest spoilage, or expressed transgenically to enhance host resistance. Because of the antibiotic, antimicrobial, and antiviral properties of the peptides, they may also be used as preservatives or sterilants of materials susceptible to microbial or viral contamination. The BGP-A or MGP-A peptides of the invention can be utilized as broad spectrum antimicrobial agents directed toward various specific applications. Such applications include use of the peptides as preservatives in processed foods (organisms including *Salmonella*, *Yersinia*, *Shigella*), either alone or in combination with antibacterial food additives such as lysozymes; as a topical agent (*Pseudomonas*, *Streptococcus*) and to kill odor producing microbes (*Micrococci*). The relative effectiveness of the peptides of the invention for the applications described can be readily determined by one of skill in the art by determining the sensitivity of any organism to one of the peptides.

It is also possible to incorporate the peptides on devices or immaterial objects where microbial growth is undesirable as a method of microbicidal inhibition or microbistatic inhibition of microbial growth in an environment capable of sustaining microbial growth by administering to the devices or immaterial objects a microbicidal or microbistatical effective amount of peptide. Such devices or immaterial objects include, but are not limited to, linens, cloth, plastics, implantable devices (e.g., heart pacemakers, surgical stents), surfaces or storage containers. Coating may be achieved by nonspecific absorption or covalent attachment.

### EXAMPLES

The following examples are intended to illustrate but not admitted to limit the invention in any manner, shape, or form (either explicitly or implicitly), nor should they be so construed. While they are typical of those that might be used, other procedures, methodologies, or techniques known to those skilled in the art may be used alternatively.

### MATERIALS AND METHODS

Bovine neutrophils. Polymorphonuclear leukocytes (PMN) were purified from 1 L batches of fresh citrated bovine blood. Following sedimentation at 40 minutes at 700×g and 37° C., the erythrocyte column was subjected to 7 seconds of hypotonic lysis, after which isotonicity was restored using 3x phosphate buffered saline. The leukocyte-rich suspension was then sedimented at 120×g (4° C., 15 minutes). Residual erythrocytes were lysed by repeating this procedure 1 or 2 times. Aliquots were removed for quantitation by hemocytometry and differential counts. Preparations obtained by this procedure contained an average of 4×10<sup>9</sup> cells per L of whole blood of which 97+3% were neutrophils. Preparations were treated with 2 mM diisopropylfluorophosphate (DFP). Neutrophil preparations were then cooled to 4° C. for 20 minutes and disrupted by nitrogen cavitation in a Parr bomb (Borregaard, N., et al., *J. Cell Biol.* 8 97:52-61, 1983). The cavitate was centrifuged at 800×g for 10 minutes at 4° C., and the granule-containing supernatant was collected. Granules were harvested by centrifugation at 27,000×g for 40 minutes and stored at -80° C.

PMN Granule extracts. Preparations of frozen granules from 1-5×10<sup>10</sup> PMN were extracted with 5 ml of ice cold

10% acetic acid per 1×10<sup>9</sup> cell equivalents. After stirring on ice for 18 hours, the suspension was clarified by centrifugation at 27,000×g for 20 minutes at 4° C. and the supernatants were lyophilized and stored at -70° C.

Size exclusion chromatography. Lyophilized granule extract was dissolved in 10% acetic acid at a concentration of ca. 1×10<sup>9</sup> cell equivalents per ml, clarified by centrifugation, and loaded onto a 4.8×110 cm column of BioGel P-60 equilibrated in 5% acetic acid. The column was run at 8° C. with an elution rate of 2 cm per hour, and 15 ml fractions were collected with continuous monitoring at 280 nm.

Reversed phase HPLC (RP-HPLC). Low molecular weight components eluting from the size exclusion column were further resolved by RP-HPLC on a Waters 510 binary system on a 1×25 cm Vydac C-18 column. Water and acetonitrile containing 0.1% trifluoroacetic acid (TFA) or 0.13% heptafluorobutyric acid (HFBA) were used for gradient elution. Purified peptides were lyophilized, dissolved in 0.01% acetic acid at 100-500 µg/ml, and stored at -70° C.

Polyacrylamide gel electrophoresis. Sodium dodecyl sulfate (SDS; 14) and acid-urea (Selsted, M. E., et al., *Anal. Biochem.* 155:270-274, 1986) gel electrophoresis were used to the estimate molecular mass and/or purity of protein preparations as previously described (Selsted, M. E., et al., *Infect. Immun.* 45:150-154, 1984).

Amino acid analysis. The amino acid composition of each peptide was determined on 6 N HCl hydrolysates (2 h, 15° C.) of native and performic acid-oxidized, or reduced and alkylated samples (Bidlemyer, B. A., et al., *J. Chromatogr.* 336:93-104, 1984). Tryptophan content was determined by sequence analysis and by spectroscopic measurement on a Beckman DU 60 spectrophotometer by the method of Edelhoch (Edelhoch, H., *Biochem.* 6:1948-1954).

Sequence Analysis. For sequence analysis, purified BGP-A was subjected to automated Edman sequence analysis. Automated sequence analysis was performed on an Applied Biosystems 475A instrument configured with on-line PTH-amino acid analysis. The sequence was confirmed by comparing the primary structure with the amino acid composition, and cDNA cloning.

Peptide synthesis. BGP-A and BGP-A-amide were synthesized at the 0.4 mmol scale on a Millipore 9050 automated synthesizer by standard Fmoc/BOP/HOBt/NMM activation with a 30 minute coupling time. The starting resin for the free acid peptide was Fmoc-L-Valine-PEG-PS (Millipore), and for peptide amide the starting resin was Fmoc-PAL-PEG-PS (Barany, G., et al., *Intercept*, R. Epton, Andover, England, 1992, pp.29-38; Van Abel, R. J., et al., *Int. J. Peptide Protein Applicant respectfully requests withdrawal of the rejection.* 45:401-409, 1995). Side chain protecting groups were Pmc for arginine, trityl for glutamine and histidine, tBoc for lysine and tBu for tyrosine. Fmoc deprotection was with 2% piperidine and 2% DBU for 15 minutes. Tryptophan and isoleucine were double coupled. Following chain assembly the resin was cleaved and deprotected with reagent K (82.5% TFA, 5% phenol, 5% thioanisole, 5% water and 2.5% ethanedithiol) for 4 hours. The peptide solution was made 30% in acetic acid, extracted with dichloromethane, and the aqueous phase was lyophilized. Purification was performed by RP-HPLC on a 22.5×250 mm preparative Nydak C-18 column using 0.1%TFA and a linear acetonitrile gradient developed at 0.33% per minute. The purified peptides were analyzed by amino acid analysis, acid-urea gel electrophoresis and analytical RP-HPLC.

cDNA isolation and characterization. BGP-A: Total RNA was isolated from bovine bone marrow using the acid guanidinium thiocyanate-phenol extraction method of Chomczynski and Sacchi (Chomczynski, P., et al., *Analyt. Biochem.* 162:156-159, 1987). Bone marrow total RNA (1 mg) was then used with avian reverse transcriptase to synthesize first strand cDNA according to the manufacturer's protocol (5'-RACE System; Life Technologies; Gaithersburg, Md.). This cDNA was used as a template for 3'-RACE, in which a degenerate gene specific primer was paired with an oligo (dT)<sub>15</sub>-anchor primer to generate the 3'-end of the BGP-A cDNA. PCR amplification was carried out using the following cycling parameters: 95° C., 1 minutes; 55° C., 1 minutes; 72° C., 1 minutes for 35 cycles. 5'-RACE was carried out in a similar fashion with the exception that first strand cDNA was tailed using terminal transferase and different gene specific and anchor primers were used. PCR-amplified RACE products were subcloned and sequenced as described previously (Yount, N.Y., et al., *J. Immunol.* 155:4476-4484, 1995). Once the 5'- and 3'-ends of the BGP-A cDNA were known, a PCR product corresponding to the full length BGP-A sequence was generated and characterized by sequence analysis.

Murine bone marrow total RNA and first strand cDNA were generated as for BGP-A. Two gene specific primers were then used to PCR amplify a sequence corresponding to a BGP-A homolog. This sequence was subcloned and sequenced as described above.

Antimicrobial assay. *E. coli* ML35, *S. aureus* 502A, *C. albicans*, and *C. neoformans* were used as target organisms in a microbicidal suspension assay as previously described (Selsted, M. E., *Genetic Engineering: Principles and Methods*, J. K. Setlow, Plenum Press, New York, 1993, pp. 131-147).

#### EXAMPLE 1

##### Purification of BGP-A

Previous electrophoretic analyses of the acid-soluble proteins of bovine PMN granules demonstrated that these preparations contain a complex mixture of proteins varying in size from 1,000 to 200,000 D (Selsted, M. E., et al., *J. Biol. Chem.* 267:4292-4295, 1992). Acetic acid extract of a granule-enriched fraction from  $1.3 \times 10^{10}$  neutrophils was chromatographed on a Bio-Gel P-60 column as described above in the section titled, "Materials and Methods." Approximately  $2 \times 10^{10}$  cell equivalents of acid solubilized granule protein was fractionated on a BioGel P-60 column and antibacterial activity in pooled eluent fractions was assayed as described in the "Materials and Methods." Fractions corresponding to Peak E were lyophilized and subjected to further purification by RP-HPLC. Each peak (A-F in FIG. 1A) contained bactericidal activity against *S. aureus* and *E. coli*. Peak F was predominantly comprising indolicidin, a novel thirteen residue antibiotic peptide amide (Selsted, M. E., et al., *J. Biol. Chem.* 267:4292-4295), and Peak E contained at least 13  $\beta$ -defensins.

Peak E fractions were combined and further purified by HPLC. One tenth of the pooled fractions from Peak E (FIG. 1a) was loaded on a 1x25 cm Vydac C-18 column equilibrated in 0.1% TFA/water (solvent A) at a flow rate of 3.0 ml/min. A linear gradient of acetonitrile (20% to 45%) containing 0.1% TFA (solvent B) was applied at the rate of 0.33% per min. Fractions were collected using the peak cutting mode of a Pharmacia Frac-200 fraction collector. The initial RP-HPLC purification of Peak E fractions

yielded a complex chromatogram (FIG. 1B) in which most peaks contained two or more peptides as determined by acid-urea PAGE. However, BGP-A was eluted as an isolated, virtually pure peak (indicated by the asterisk symbol "\*" in FIG. 1B) early in the RP-HPLC chromatogram. Final purification (FIG. 2) was obtained by a second round of RP-HPLC.

#### EXAMPLE 2

##### Amino Acid and Sequence Analysis of BGP-A

The composition of BGP-A was established by amino acid analysis (FIG. 2). Approximately 5  $\mu$ g of purified BGP-A was injected onto a 0.4x25 cm Vydac C-18 column run at a flow rate of 1.0 ml/min. Solvents are the same as described above for FIG. 1B. Gradient conditions: 10% B to 50% B in 25 min. B. Acid-urea gel of purified BGP-A. A 2  $\mu$ g sample of purified BGP-A was loaded onto a 12.5% acid-urea polyacrylamide gel that was electrophoresed for 4 hours at 250 V (lane 2). A 100  $\mu$ g sample of crude acid extract from bovine neutrophil granules (lane 1) was run in parallel. Staining was with Coomassie Blue containing 15% formalin. Absorbance scans of BGP-A were carried out between 300 and 200 nm, providing an accurate estimate of tyrosine and tryptophan content (Edelhoch, H., *Biochem.* 6:1948-1954, 1967). Automated sequence analysis was carried out on 2 nmol of BGP-A. Repetitive sequencing yields averaged  $\geq 90$  percent, allowing for unambiguous assignment of all thirteen residues. The complete amino acid sequence of BGP-A is:

Tyr-Lys-Ile-Ile-Gln-Gln-Trp-Pro-His-Tyr-Arg-Arg-Val  
(SEQ ID NO: 5; FIG. 6)

A protein sequence search using the BLAST algorithm (Altschul, S. F., et al., *J. Molec. Biol.* 215:403-410, 1990) revealed no similar amino acid sequences among the GenBank Data base.

#### EXAMPLE 3

##### Synthesis of BGP-A and BGP-A-Amide

The two synthetic BGP-A forms were assembled as N<sup>α</sup>-Fmoc protected amino acids. (The acid-urea gel patterns of the purified peptides are shown in FIG. 3.) A 12.5% acid-urea gel was loaded with 2-4  $\mu$ g of natural BGP-A (FIG. 3, lane 1), synthetic BGP-A (FIG. 3, lane 2) or synthetic BGP-A-amide (FIG. 3, lane 3). Staining was as described for FIG. 2. The yields of the HPLC-purified material were 31.4% for the free acid form, and 22.1% for the carboxamidated form.

#### EXAMPLE 4

##### Isolation and Sequencing of BGP-A cDNA Clones

The full length BGP-A cDNA is 688 nucleotides in length (SEQ ID NO: 2) and predicts a 21 kD precursor composed of 190 residues (FIG. 4; SEQ ID NO: 3). Within the BGP-A precursor, 11 of the first 21 residues are hydrophobic and predict a signal peptide (Von Heijne, G., *Eur. J. Biochem.* 133:17-21, 1983). The signal peptide domain is followed by an intervening propeptide region containing 156 residues. The final 13 residues of the precursor correspond to the mature BGP-A peptide sequence (SEQ ID NO: 6).

To determine if the BGP-A precursor was homologous to other nucleotide or protein sequences, a Blast search of the GenBank database was carried out. Some homology between the BGP-A sequence and a partial cDNA sequence

isolated from murine adenocarcinoma of unknown tissue origin was identified. Using consensus primers derived from the murine adenocarcinoma and BGP-A sequences, a cDNA encoding a BGP-A like sequence from mouse bone marrow (FIG. 5; SEQ ID NO: 5) was isolated. This full-length cDNA is 679 nucleotides in length (SEQ ID NO: 4) and predicts a precursor comprising signal pro-peptide domains similar to those described for BGP-A (FIG. 5; SEQ ID NO: 5). The mature peptide sequence predicted by the murine cDNA is identical to BGP-A at 7 of 13 residues (FIG. 6; SEQ ID NO: 7). Based on this similarity, this sequence isolated from murine bone marrow cDNA is designated as mouse granulocyte peptide A (MGP-A; FIG. 5; SEQ ID NO: 5 and FIG. 6, SEQ ID NO: 7).

#### EXAMPLE 5

##### Antimicrobial Activity of BGP-A and BGP-A-Amide

Natural and synthetic BGP-A and synthetic BGP-A-amide were tested for their microbicidal activities against *S. aureus* 502A, *E. coli* ML35, *C. albicans*, and *C. neoformans*. Using a microbicidal suspension assay (Selsted, M. E., *Genetic Engineering: Principles and Methods*, J. K. Setlow, Plenum Press, New York, 1993, pp.131-147), each peptide was tested against the four test organisms with peptide concentrations ranging from 5-100  $\mu$ g/ml. The bactericidal and fungicidal activities of the three peptide preparations were assessed using a standard microbicidal assay. Organisms were grown to mid-log phase, harvested, and suspended to  $2 \times 10^7$  CFU/ml. The incubation mixture contained  $1-2 \times 10^6$  CFU/ml, 10 mM sodium phosphate buffer, pH 7.4, and peptide at concentrations up to 100  $\mu$ g/ml. After 1 h of incubation at 37° C. (4 h incubations for *C. neoformans*), serial 10-fold dilutions were plated on Trypticase Soy Agar (bacteria) or *S. abaraud* dextrose agar (fungi), and incubated for 24-48 h at 37° C. Killing was quantitated by colony counting, and plotted as a function of peptide concentration in the incubation.

The data, presented in FIG. 7, reveal the dose-dependent activity of each peptide as measured by the reduction in colony forming units after a 1 or 4 hour incubation interval. These data demonstrate 1) that BGP-A was microbicidal against each organism; 2) that synthetic BGP-A and natural BGP-A were equal in potency, suggesting that the activity of the natural peptide was attributable to the purified compound and not to a contaminant; and 3) that the carboxamidated form of BGP-A is much more potent against most of the targets than is the free-carboxyl form.

The mature peptide was microbicidal in vitro against representative Gram positive and Gram negative bacteria, and yeast forms of two fungi. The antimicrobial activity of the natural peptide was validated by demonstration that synthetic BGP-A had equivalent killing activity.

#### EXAMPLE 6

##### Activity of BGP-A and BGP-A-Amide to Treat an LPS Disorder

The effect of the BGP, MGP, BGP-A and MGP-A peptides of the invention on LPS-induced TNF in macrophages can be determined by those in the art, according to standard methods. For example, macrophage cells are grown by seeding cells into a cell culture flask and incubated at 37° C., 5% CO<sub>2</sub> for 1 week. Macrophage cell media [(Dulbecco's Modified Eagle Medium with Hepes buffer 450 ml; 2.4 mM

L-glutamine 3 ml (400 mM); Pen/Strep 3ml (10<sup>4</sup>U/ml of Pen, 1 mg/ml strep); and 10% heat inactivated fetal bovine serum (FBS) 50 ml]] is then completely removed from flasks. 10 mls of cell dissociation solution (Sigma) is added to each flask and incubated at 37° C. for 10 minutes. Cells are removed from flasks, diluted in macrophage cell media and centrifuged for approximately six minutes. The cell pellet is resuspended in 5 ml of media/flask used. 100  $\mu$ l cell suspension is removed and added to 400  $\mu$ l of trypan blue and cells are counted using a hemocytometer. The cell suspension is diluted to  $1 \times 10^6$  cells/ml and 1 ml of suspension is added per well of a 24 well plate. The 24 well plates are incubated at 37° C., 5% CO<sub>2</sub> overnight.

After an overnight incubation, the media is aspirated from all the wells. 100  $\mu$ l of Lipopolysaccharide (LPS) is added at 100 ng/100  $\mu$ l. BGP-A and MGP-A is added at the desired concentration/100  $\mu$ l to specified wells. Macrophage cell media is added to a final volume of 1 ml/well. The plates are incubated for six hours at 37° C., 5% CO<sub>2</sub>. The supernatant is removed from the wells and stored overnight at 4° C. For those wells in which whole bacteria is added directly to the wells, the supernatant is centrifuged in 0.2  $\mu$ m filter eppendorf tubes for 5 minutes.

The supernatants are then used in cell cytotoxic L929 assay. The samples are transferred to 96 well plates. 50  $\mu$ l of TNF media is added to all the wells in all the plates except to those wells in the first row. 10  $\mu$ l of murine TNF standard (20 ng/ml) and 90  $\mu$ l of TNF media is added in duplicate to the plate and diluted 1:2 down the plate to the second to last row. Test samples (75  $\mu$ l), comprising the supernatants from the macrophage cell assays, are added to separate rows in duplicate and diluted 1:3 to the second to last rows.

TNF-sensitive L929 mouse fibroblast cells are grown by seeding  $10^6$  cells into a 162 cm<sup>2</sup> cell culture flask and left to grow for 1 week, L929 cells are removed from the flask with 10 mls of trypsin-EDTA/flask and incubated 3-5 minutes. Cell suspension is diluted and centrifuged for 6 minutes. The pellet is resuspended in 5 mls of fresh L929 media/flask and counted (same as macrophage cells). Cell suspension is diluted to  $10^6$  cells/ml. 100  $\mu$ l is used to inoculate each well of the 96 well plates with the supernatants. (L929 Growth Media is the same as macrophage cell media except instead of FBS, 50 mls of 10% heat inactivated horse serum is utilized; TNF Assay Media is the same as macrophage cell media except 4  $\mu$ g/ml Actinomycin D is added.)

The plates are incubated at 37° C. at 5% CO<sub>2</sub> for 2 days. The media is then aspirated and replaced with 100  $\mu$ l of the dye MTT (0.5 mg/ml) in modified Eagle Medium without phenol red. The plates are then incubated at 37° C. at 5% CO<sub>2</sub> for 3 hours. The dye is then removed and replaced with 100  $\mu$ l of absolute ethanol. The plates are left at room temperature for 10-15 minutes to dissolve the formazan dye crystals.

The plates are read at 570 nm in a ELISA plate reader with 690 nm reference filter. One unit of TNF activity is defined as the amount required to kill 50% of the L929 cells. The TNF level in Units per ml therefore is the reciprocal of the dilution which led to a 50% killing of L929 cells.

It is to be understood that, while the invention has been described with reference to the above detailed description, the foregoing description is intended to illustrate, but not to limit, the scope of the invention. Other aspects, advantages, and modifications of the invention are within the scope of the following claims. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 7

## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

(D) OTHER INFORMATION: Xaa at amino acid residue 2, 3, 6 or 8 can be = Ala, Arg, Asn, Asp, Cys, Glu, Gln, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr or Val.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Tyr Xaa Xaa Ile Gln Xaa Trp Xaa His Tyr Arg  
 1 5 10

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 688 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

AGTCTCCGCG TGTCTTTTCC TGCCTGCC ATG TCT CGC CGC TAC ACA CCG CTC 52  
 Met Ser Arg Arg Tyr Thr Pro Leu  
 1 5

GCC TGG GTC CTC CTC GCC CTC CTG GGC CTC GGG GCG GCT CAA GAC TGC 100  
 Ala Trp Val Leu Leu Ala Leu Leu Gly Leu Gly Ala Ala Gln Asp Cys  
 10 15 20

GGC AGC ATC GTG TCC CGC GGA AAG TGG GGC GCC CTG GCA TCC AAG TGC 148  
 Gly Ser Ile Val Ser Arg Gly Lys Trp Gly Ala Leu Ala Ser Lys Cys  
 25 30 35 40

AGC CAG AGG CTA AGA CAG CCT GTG CGC TAC GTG GTG GTG TCG CAC ACG 196  
 Ser Gln Arg Leu Arg Gln Pro Val Arg Tyr Val Val Val Ser His Thr  
 45 50 55

GCG GGC AGC GTC TGC AAC ACT CCG GCC TCG TGC CAG AGG CAG GCC CAA 244  
 Ala Gly Ser Val Cys Asn Thr Pro Ala Ser Cys Gln Arg Gln Ala Gln  
 60 65 70

AAC GTG CAG TAC TAC CAC GTG CGG GAG CGG GGC TGG TGC GAC GTG GGC 292  
 Asn Val Gln Tyr Tyr His Val Arg Glu Arg Gly Trp Cys Asp Val Gly  
 75 80 85

TAC AAT TTC CTG ATC GGA GAA GAT GGG CTC GTG TAT GAG GGC CGG GGC 340  
 Tyr Asn Phe Lys Ile Gly Glu Asp Gly Lys Val Tyr Glu Gly Arg Gly  
 90 95 100

TGG AAC ACC TTA GGT GCT CAC TCT GGG CCC ACG TGG AAC CCC ATA GCC 388  
 Trp Asn Thr Lys Gly Asp His Ser Gly Pro Thr Trp Asn Pro Ile Ala  
 105 110 115 120

ATC GGC ATC TCC TTC ATG GGC AAC TAC ATG CAT CGG GTG CCC CCG GCC 436  
 Ile Gly Ile Ser Phe Met Gly Asn Tyr Met His Arg Val Phe Phe Ala  
 125 130 135

TCT GCT CTC AGG GCG GCC CAG AGT CTG CTG GCT TGT GGC GCA GCT CGG 484  
 Ser Ala Leu Arg Ala Ala Gln Ser Leu Leu Ala Cys Gly Ala Ala Arg  
 140 145 150

-continued

GGA TAC CTG ACT CCT AAC TAC GAA GTC AAA GGA CAC CGC GAT GTG CAG 532  
 Gly Tyr Leu Thr Pro Asn Tyr Glu Val Lys Gly His Arg Asp Val Gln  
 155 160 165

CAG ACG CTC TCT CCA GGG GAC GAG CTC TAT AAA ATC ATC CAG CAG TGG 580  
 Gln Thr Leu Ser Pro Gly Asp Glu Leu Tyr Lys Ile Ile Gln Gln Trp  
 170 175 180

CCG CAC TAC CGC CGC GTG TGAGGGCCTG TCCGTCTTCT CACACCCAC 628  
 Pro His Tyr Arg Arg Val  
 185 190

CCATCCCATC AGAAACCCCA CGCCTTCCC CTGCCCAAT AAAGGCGAAG CTTAAACTGT 688

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 190 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 39..598

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Ser Arg Arg Tyr Thr Pro Leu Ala Trp Val Leu Leu Ala Leu Leu  
 1 5 10 15

Gly Leu Gly Ala Ala Gln Asp Cys Gly Ser Ile Val Ser Arg Gly Lys  
 20 25 30

Trp Gly Ala Leu Ala Ser Lys Cys Ser Gln Arg Leu Arg Gln Pro Val  
 35 40 45

Arg Tyr Val Val Val Ser His Thr Ala Gly Ser Val Cys Asn Thr Pro  
 50 55 60

Ala Ser Cys Gln Arg Gln Ala Gln Asn Val Gln Tyr Tyr His Val Arg  
 65 70 75 80

Glu Arg Gly Trp Cys Asp Val Gly Tyr Asn Phe Lys Ile Gly Glu Asp  
 85 90 95

Gly Lys Val Tyr Glu Gly Arg Gly Trp Asn Thr Lys Gly Asp His Ser  
 100 105 110

Gly Pro Thr Trp Asn Pro Ile Ala Ile Gly Ile Ser Phe Met Gly Asn  
 115 120 125

Tyr Met His Arg Val Phe Phe Ala Ser Ala Leu Arg Ala Ala Gln Ser  
 130 135 140

Leu Leu Ala Cys Gly Ala Ala Arg Gly Tyr Leu Thr Pro Asn Tyr Glu  
 145 150 155 160

Val Lys Gly His Arg Asp Val Gln Gln Thr Leu Ser Pro Gly Asp Glu  
 165 170 175

Leu Tyr Lys Ile Ile Gln Gln Trp Pro His Tyr Arg Arg Val  
 180 185 190

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 628 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: Genomic DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

-continued

ATACACAGCC CTGCGTCTGT TCGGGCACGT CCAGC ATG TTG TTT GCC TGT GCT	53
Met Lys Phe Ala Cys Ala	
1 5	
CTC CTT GCC CTC CTG GGT CTG GCA ACC TCC TGC AGT TTC ATC GTG CCC	101
Leu Leu Ala Leu Leu Gly Leu Ala Thr Ser Cys Ser Phe Ile Val Phe	
10 15 20	
CGC AGT GAG TGG AGG GCC CTG CCA TCC GAG TGC TCT AGC CGC CTG GGG	149
Arg Ser Glu Trp Arg Ala Leu Pro Ser Glu Cys Ser Ser Arg Leu Gly	
25 30 35	
CAC CCA GTT CGC TAC GTG GTG ATC TCA CAC ACA GCC GGC AGC TTC TGC	197
His Pro Val Arg Tyr Val Val Ile Ser His Thr Arg Gly Ser Phe Cys	
40 45 50	
AAC AGC CCG GAC TCC TGT GAA CAG CAG GCC CGC AAT GTG CAG CAT TAC	245
Asn Ser Phe Asp Ser Cys Glu Gln Gln Ala Arg Asn Val Gln His Tyr	
55 60 65 70	
CAC AAG AAT GAG CTG GGC TGG TGC GAT GTA GCC TAC AAC TTC CTT ATT	293
His Lys Asn Glu Leu Glu Trp Cys Asp Val Ala Tyr Asn Ile Lys Glu	
75 80 85	
GGA GAG GAC GGT CAT GTC TAT GAA GGC CGA GGC TGG AAC ATC AAG GGT	341
Asp His Thr Glu Pro Ile Tyr Asn Pro Met Ser Ile Gly Ile Thr Phe	
90 95 100	
ATG GGG AAC TTC ATG GAC CGG GTA CGC AAA GCG GCC CTC CGT GCT GCC	389
Met Gly Asn Phe Met Asp Arg Val Arg Lys Ala Ala Leu Arg Ala Ala	
105 110 115	
CTA AAT CTT CTG GAA TCT GGG GTG TCT CGG GGC TTC CTG AGA TCC AAC	437
Leu Asn Leu Leu Glu Ser Gly Val Ser Arg Gly Phe Leu Arg Ser Asn	
120 125 130	
TAT GAA GTC AAA GGA CAC CGG GAT GTG CAA AGC ACT CTC TCT CCA GGT	485
Tyr Glu Val Lys Gly His Arg Asp Val Gln Ser Phe Leu Ser Phe Gly	
135 140 145 150	
GAC CAA CTC TAT CAG GTC ATC CAA AGC TGG GAA CAC TAC CGA GAG	530
Asp Gln Lys Tyr Gln Val Ile Gln Ser Trp Glu His Tyr Arg Glu	
155 160 165	
TGAGAGACCT TGAGACCTAG TGAGAATCCC CCCCCCAGC CCGAAATCCC TCCTGCCACC	590
TGCTTCTTCC CATTGACCCC CAATAAGAC TCAGCACC	628

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 165 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 36..521

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Lys Phe Ala Cys Ala Leu Leu Ala Leu Leu Gly Leu Ala Thr Ser	
1 5 10 15	
Cys Ser Phe Ile Val Phe Arg Ser Glu Trp Arg Ala Leu Pro Ser Glu	
20 25 30	
Cys Ser Ser Arg Leu Gly His Pro Val Arg Tyr Val Val Ile Ser His	
35 40 45	
Thr Arg Gly Ser Phe Cys Asn Ser Phe Asp Ser Cys Glu Gln Gln Ala	
50 55 60	
Arg Asn Val Gln His Tyr His Lys Asn Glu Leu Glu Trp Cys Asp Val	
65 70 75 80	



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